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PROGRESS

SOCIAL REVOLUTION AND THE PHYSICIAN*

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LET us for a moment turn the calendar back eight short years, to the year of our Lord 1933.

This was a year of depression. The banks took a holiday. Even so, we were making progress. The eighteenth amendment was repealed, destroying the problem of racketeers. The Industrial Recovery Act was passed, assuring prosperity. The bonus grab was consummated, distributing billions of dollars, that our country might lift itself by its bootstraps. Chicago was celebrating a Century of Progress. Wiley Post was circling the world in eight days. The Golden Gate Bridge was abuilding and the first streamliner train was entering service. Science and medicine were making strides. Heavy water was being discovered; magic eyes were opening doors. Surgeons were removing entire lungs. The Rockefeller Foundation was spending fifteen million dollars to promote the well-being of mankind.

All was reasonably well with the world.

On January 30 a fiery little fanatic, Adolf Hitler, became Chancellor of Germany. Within the year Mower wrote a book, *The Brown Book of the Hitler Terror*. Armstrong wrote another, *Germany Puts the Clock Back*. The Nobel Peace Award for 1933 was postponed.

Will that year go down as the nineteen hundred thirty-third from the birth of Christ? Or will it gain more lasting place as the year "One" of the New Dark Ages?

There have been glorious epochs in history. A high point was the Age of Pericles, when commerce and industry, art, philosophy, and the science of the

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time flourished. We read of the rulers and sages of that age, and call it Golden. We forget that its average persons lived as crudely as the barbarians who later destroyed them. We forget that the glories of ancient Greece were built upon a foundation of slavery.

If I had my choice of any half century in which to live during the last fifty thousand years, I would select the years just elapsed and would request life in a democracy, preferably in the United States. At no other time or place in the history of creation have men, from the wealthiest and the most intelligent to the poorest and the least intelligent, enjoyed comparable comfort and freedom.

True, this period has witnessed disease and crime. Utopia is not yet here, but it seemed almost within reach. At what point on the road of progress did our race stray into the bypath which has led us to the brink of disaster?

During the first World War a new element entered those factors which appeared to be controlling progress. A few recognized this at the time. Many do so now when it is almost too late. This new element is complex. It is a trend, best analyzed in the changes in social psychology since the last war.

In the intervening decades there has been continuous advance in the physical or mechanical aspects of living. Automobiles and ships have improved. Diesel trains, air-conditioned buildings, and other inventions have made living almost too comfortable. Scientific achievement is gaining, not losing, momentum. New discoveries are now heralded daily, and the science and the art of medicine are at a high point of achievement.

In these aspects the world appears to have been traveling onward toward grander things. The downward change has been in our atttiude toward life, in our interpretation of the reason for existence, and in our evaluation of those things for which life is worth the living. I might say that in a mechanized world, our philosophy has become mechanistic.

Many factors have played a part. The first, a direct outcome of World War I, was man's unconscious denial of the value of human life. Only by the adoption of such an attitude can soldiers live through a war of destruction without going insane. When one sees dear friends, stalwart young men, blown to fragments, knowing that the next shell may be his own, one forgets the platitudes of the Sunday-school teacher on God's interest in the weakest lamb of the flock. The success of wars depends upon mass training in the psychology of the unimportance of the individual. All of us who were in France absorbed it, whether we were fighting with bayonets or merely closing the eyes of others as they died from violence or disease.

Such a psychologic attitude persists after its need has passed.

A second factor which in this country has changed the outlook of the masses was prohibition, which made gentlemen out of drunkards and drunkards of gentlemen; which allowed honest men to deal with crooks; which made connivers of policemen and bars into speakeasies; and which bred contempt of the law in every class, but most regrettably in children, as they watched their parents flout it and laugh.

I find a third conditioning influence in the present trend toward mediocrity in productive effort, especially among the laboring groups. Time was when craftsmen and their apprentices derived as much satisfaction from the making of an artistic copy of a famous statue, painting, saddle, nutcracker, or bookmark as the artist had in his original creation. Today, the trade unions discourage originality. All must be stereotyped. No matter what the degree of one's ability, all must work the same hours and receive the same pay. Creative ambition among craftsmen is discouraged. Once there were cabinet makers. Then there were carpenters. Now there are saw-and-hatchet men who piece together jigsaw puzzles of prefabricated materials.

These three influences, mediocrity, lawlessness, and negation of the importance of the individual, have probably been the most important factors in this country. The trend has been world wide. It is illustrated in the communism of Russia and the corrupt politics of France.

There is a fourth factor which has influenced nations. While Germany has been the leader in this factor, it has been reflected in her allies, Japan, Italy, and the Russia of 1940. It may be summarized in the term national dishonesty. If a government lies, cheats, steals, and murders, the ethics of those living under it must of necessity undergo subtle changes.

We are living in a period of world revolution, of turmoil, in which the socialistic trend, the trend toward mediocrity, the tendency to draw the best down toward the worst is dominant. We have seen civilizations fall. Is today's situation comparable to the softening that caused the downfall of Rome? Are we witnessing a temporary eclipse of civilization?

For myself, the answer is no. But I must qualify this answer.

We are entering a new world order, one which may not make the world better. We are in a phase comparable to the French Revolution. It involves a reshuffling in which the "haves" will become "have nots," but the "have nots" will not become "haves." The outstanding phenomenon of the catastrophe is the destruction of individualism. Individualism was destroyed in the French Revolution, but it returned. It will always return.

How have we managed to get ourselves into this predicament? Have we taken a byway off the path of progress? Or have we reached a previously uncharted area on the main road? Are there more basic conditioning elements than the superficially obvious ones which I have described? I see three such possibilities.

The first is the influence of cosmic forces. The suggestion has been made that those solar convulsions which cause sun spots may extend their influence to things living on earth. It has been shown that periods of social unrest coincide fairly well with phases of increase in sun spots. This applies to the World War, the Russian Revolution, and even to runs on the stock market. When William Petersen is showing how terrestrial weather may influence man physically, mentally, and emotionally, we need not stretch imagination to grant the possibility that cosmic rays or other solar forces might influence our activities. When C. A. Mills tells us that the upward trend in college student stature appears to be undergoing a reversal, and that sexual maturity is occurring at

a later age; when he attributes this to the unseasonable warmth prevailing over the earth since 1929; and when he remarks that the physical recession of mankind during the Dark Ages occurred during centuries of world warmth, we must ponder on the possible influence of cosmic forces on human progress.

We turn from such conjectures, since their acceptance implies a defeatist attitude. But we cannot ignore them if they may be true. If we grant their possibility, then our every effort should be to counteract such deterministic tendencies with whatever means science may make available.

We must consider human factors as well as geoastral forces. There are two: the growth of science, and the failing influence of religion.

The Christian religion has been the greatest social force for good that the world has ever known. I say this with full realization of the hypocrisy, injustice, corruption, torture, and brigandage that have been committed in the name of Christ. The Jewish, Buddhist, and Confucian religions have been comparably potent for good, although probably less widely effective. A single discovery nearly 400 years ago blasted the tenets of all theologies. When Copernicus proved that the earth revolves about the sun, our planet could no longer be the center of the universe. It became just a little ball, gyrating in unison with countless other little balls.

Men of science have been forced by their discoveries to become agnostic. They find no proof of individual life hereafter. They have adjusted themselves to the philosophy of contributing greatest good for the greatest number on this earth, and to the knowledge of immortality at least in the germ plasm of one's descendants. Surely we can speak of the religion of science. Members of this cult are today leading a more ethical life than are some so-called pillars of the church, even though the former have no fear of eternal damnation or hope of reward in the hereafter. In this way, the advanced thinkers have solved their problem. The trouble is that, with the popularization of science, nonthinkers have also become convinced that there is no hereafter. The great controlling force of orthodox religion over men with antisocial tendencies has been the fear of hell fire. With this gone, potential criminals are all out for what they can get in this life. Destruction of the concept of immortality, brought about by science, has been a potent factor in increasing crime on this earth.

We are living in days of realism. This applies even in the young. A five-year-old Jewish boy had heard his parents discuss the tragedy of the Jews in Germany. One day, disobeying injunctions, he went to the river bank where, of course, he fell in. His Mother told him that God, angry at his disobedience, had punished him. Next time God might let him drown. To which the lad replied, "I hate God." His reasoning was logical: "Why should God do that to me, when he does nothing to Hitler?"

The deity myth is one of the most remarkable phenomena in history. Anthropomorphic God failed his only Son two thousand years ago. Did we accept this as evidence of nonexistence? No, we made excuses, we explained that he wanted Christ to suffer like man. And this man-made illusion has continued

to govern the world for two millennia, fortunately so, because we were not ready for the next step in human evolution.

It has been said that the one hope for dogmatic religion is the arrival of a new Messiah. He must come from the lowest classes. He must fire the imagination. His religion must be militant. It must offer promise of heaven on earth. Today some see such a Messiah. His name is Adolf Hitler. He is the most evil genius that the forces of creation have ever let loose upon this earth.

What may we expect from science? Scientists have realized that average man must have a tangible religion. To this extent they have regretted the passing of the old. Some have attempted to substitute a religion of ethics based upon science. Their writings usually start convincingly and end in a philosophic hodgepodge which leaves their readers and, I suspect, themselves in a complete fog, proving that the scientist should limit himself to science and leave metaphysics and philosophic dissertations to those better qualified in the field.

One writer, Edwin Conklin, has avoided the pitfalls. In his essay "Does Science Afford a Basis for Ethics?" he formulates the answer more clearly than any other whose writings it has been my pleasure to read. Briefly excerpted, his reasoning is as follows.

"There is evidence that in times long past there were types of human beings, more brutish in body, mind and social relations than the general average of the present race. There is abundant evidence that ethics has undergone evolution no less than intelligence. It seems incredible that reason, emotion, aspiration and ethics should develop out of such simple functions as sensitivity, reflexes, trial and error, and yet these incredible things are actual facts.

"Psychical development begins with the ability to respond differently to stimuli of different quality or degree. Just as the amoeba avoids extremes of hot or cold, all living things grow toward the satisfactory and avoid the unsatisfactory. This is the beginning of psychic life. It is based upon the ability to distinguish between that which is satisfactory and that which is not, and to follow after the one and to avoid the other. This very fact of distinguishing and choosing or following is the basis of wisdom. Freedom comes with the ability to make a choice between two alternatives, and is measured by the extent to which remembered experience influences behavior. Intelligence is the capacity of profiting by remembered experiences.

"The highest level of human development is attained when purpose and freedom joined to social emotion, training and habits, shape behavior not only for personal but also for social satisfaction, since society no less than the individual is seeking satisfaction. When all these things combine, we have what we call ethics or the science of right conduct.

"The process of evolution involves not only the physical body but mental attributes and ethical reactions. Ethical or social reactions can be measured in groups of laboratory animals as easily as physical attributes. Evolution occurs in the body, the mind, and in social relations.

"Among savage tribes ethics or altruism pervades the tribe but does not extend beyond. In the higher social grades altruism reaches farther and takes in more people, until with some persons it includes the whole human race."

Cyril James has defined society as "an organism which we can envision as endowed with a kind of independent life that enables it to continue in existence despite the procession of births and deaths that determines the lives of the men and women who compose it."

We may gather several inferences from these statements. "Survival of the fittest" does not apply only to brute force. The prize fighter is not best fitted to fly a plane, to operate a submarine, or to organize an ideal social group. In evolution the highest men have developed not only physically and mentally but also socially or ethically.

The distinctive attribute of Jesus was that he was from 2,000 to 4,000 years ahead of his time. In the evolution of man, as far as ethics is concerned, he was precocious. There were others such as Moses and the Greek philosophers. With increasing numbers in later centuries, progressively fewer stand as far above their fellow men.

Socially, Christ was a perfect man. Plato was another, of almost comparable stature. His "Republic" is still the ideal to be attained, although it may be another two thousand years before we reach it.

There is another inference that I would draw from Conklin's essay. In the process of evolution among social organizations the democracy is farther advanced than any other form of government. The difficulty with the democracy of today is that, although it has a thousand superior ethical thinkers to one superior thinker of two thousand years ago, the mass of its members is not yet sufficiently evolved. When evolution has proceeded to that stage where all have acquired ideal ethics, then democracy will be perfect. I believe that time will come.

A corollary is that the Germanic tribes of today are not at as high a stage of evolution as the English, the Scandinavian, the American, and those other political communities which have demonstrated their capabilities for self-government. The German people have no genius for this. We blamed Wilhelm in 1914. We blame Hitler now. If there had been no Kaiser or no Hitler there would have been someone else. The trouble is with the mass psychology of the Teutonic race as much as it is with its rulers. The ethos, the code of ethics of the democratic states, is one of "give and take." The Nazi code is simply that of "take."

Our error at the last peace was the assumption that democratic government would be appropriate for men who were insufficiently evolved to handle it. If the democracies win this war a benevolent authoritarian government must be set up for Germany.

I find food for thought in the observation that while the Celts and then the Teutons first settled northern Europe, the Saxons and Angles broke away from the latter, to inhabit the British Isles. These could not tolerate a static life any more than their descendants who colonized America. Those who pushed always farther into the unknown where they could develop new and better social organizations may have been those farthest advanced in evolution. If we accept the cradle of the Aryan race as in Western Asia, and compare the Slavs, the

Teutons, and the Anglo-Saxons, we may conclude that the farther they have gone geographically the better has been their capacity for self-government.

I do not wish to imply that the democracies have a monopoly of the finest minds. There have been superior ethical men in Germany, also in the Slavic nations and elsewhere. Many in Germany have been Jews, but some have been Teutons. The fact remains that when the Mediterranean races were in the flower of civilization, the Germanic tribes were still barbarians. For all of Hitler's vaunting of his personal group of Aryans, and in spite of the many fine minds that have lived in Germany—and escaped from Germany—compared with the most highly evolved groups in the world today, those who rule Germany now are one thousand years behind. They are still in the period of rule by deceit, cunning, and might. Their social ethics has not yet matured.

When Hitler entered Poland he told his people that he had taken a step which would change the history of the world for a thousand years. If he wins, this will be true. Human progress will have to step back one thousand years, and hope that a thousand years hence it will be back where it now is. I say this advisedly. Hitler's policy is one of destruction of the thinkers, the progressives, the ethical. During the two thousand years since Christ we have reached that stage where there is an appreciable, almost a militant, proportion of ethical persons. With their destruction, the rule of the barbarian will be complete, and the clock will have been set back ten centuries.

What will this world be like a thousand years from now? Kirtley Mather tells us that the present supply of oil in the United States will be exhausted in thirty years; the known world's supply, within seventy-five years. We have enough coal to last two thousand to three thousand years. Science can now make gasoline and similar products from coal. But there will be an increasing demand for nonrenewable products, such as coal and metals. Mather estimates that the demand will be doubled within a few decades. There will also be an increase in population. He concludes, "Taking all these things into consideration it would appear that world stores of needed natural resources are adequate to supply a basis for the comfortable existence of every human being who is likely to dwell anywhere on the face of the earth for something like a thousand years."

May I, for the sake of rhetorical effect, stretch my imagination and ask you to stretch yours to visualize a Hitler victory which will set the world back a thousand years; which will result in colossal wastage of nonreplaceable resources as is occurring today in the sinking of ships, the destruction of property, and the wanton explosion of those things which should be used by superior man in his daily life, ending a thousand years from now in the bankruptcy of our resources and the extinction of the human race?

Mather tells us that species like the dinosaurs, which have become extinct, lived an average of half a million years before they vanished. *Homo sapiens* has had only fifty thousand years of existence. Must we let Hitler and his minions rob us of four hundred and fifty thousand years?

Thirty years ago Prussian scientists were boasting racial superiority. Today it is Aryan purity. Then, it was superman. The superman of today is less

common among the Germans than in the more socially integrated democracies. Arrogance, intolerance, and sadism are not his characteristics.

During the era of splendid German scientific achievement, around the turn of the century, they were so positive, so dogmatic, so intolerant of dissension that they convinced not only themselves but many others of their intellectual superiority. After several decades the clay feet commenced to show. I was amused recently by the writings of some leading Germans regarding the causes of hay fever and asthma. Their conclusions of 1900 were as wrong as they could be. Today we know that hay fever is not a bacterial infection, and that asthma is not primarily a neurosis, but if you were to read those forty-year-old essays today and were not acquainted with progress in the intervening period, you would be as convinced as they were of the infallibility of their conclusions.

No, superman will not be defined in terms of the physical or mental prowess of the individual, but rather in his adaptation as a unit in the social structure. Christian kindliness, not arrogance, will be his outstanding characteristic. He will not hesitate to fight if necessary, but only for truth and justice. Even Jesus drove the money changers from the temple. I say *Christian* kindliness because Christianity is still an ideal religion. Take away the concept of the supernatural which was added after Christ's death. Take away the promise of personal immortality. What have you left? We still have the keystone—the teachings of Christ—a creed of moral and social ethics which has no superior.

We still have the Golden Rule.

The Prussian's conceit almost convinced us that his was the race of supermen. We are startled to discover that with the rationalization of science we ourselves have better claim to this distinction. If this is true, we have a heavy obligation, the task of preserving the higher civilization from destruction by those less advanced races which have assumed a camouflaging veneer of culture and are now using the implements of science, devised for social progress, for destroying society.

Here are you young men and women, at the beginning of your life's career, full of enthusiasm and ambition, and the feeling that all is reasonably well with the world. And here am I, an apostle of semigloom, invited here to wish you Godspeed and success. By way of apology and explanation may I say to you that I have four sons, all headed for careers in medicine. As a father, world problems have given me deep concern. I can only say that the advice I am giving you is the advice that I have given my own sons.

There have been other purveyors of gloom. The man who said "there is scarcely anything around us but ruin and despair" was William Pitt, the date 1800. The Duke of Wellington on the eve of his death in 1851 "thanked God that he could be spared from seeing the consummation of ruin that was gathering around." Disraeli ten years earlier had written, "In industry, commerce and agriculture there is no hope." Lord Shaftesbury said in 1848, "Nothing can save the British Empire from shipwreck." A hundred and forty years ago Thomas Jefferson wrote that dictators will "destroy every trace of civilization in the world and force mankind back into a savage state."

Today it seems truer. It may be because of our proximity or because the social upheaval is more world wide. Undoubtedly the future will be different.

What are you young men and my sons and I and the rest of us to do about it? I can give you only the high points.

First, you must prepare yourselves. Some of you are about to finish your medical education, others are just beginning. There will be a shortage of doctors and you will aid in keeping this at a minimum.

Next, your function will be to help win the war, for I can assure you it is our war, a battle against extinction of the most highly developed groups of the human race. Once you are in it, war is not a horrible affair. It is a thrilling experience. Otherwise it would not have been so popular through the ages. There is a mass psychology which destroys the sense of fear. You may be killed; if so, you will not remember it. You may be maimed, and this is the sad part of war; in the present barbaric struggle this applies equally to noncombatants. The chances are better than even that you will come through unscathed. There will be hardships, possibly heartbreaks, but we who went through the last war know that these are speedily forgotten and that in retrospect, and indeed in the day-to-day experience, fighting is lots of fun.

After we have won the war or, God forbid, lost it, what then? Present trends presage increasing socialization, involving scientists and physicians with the rest of humanity.

May I present three general alternatives? First, you can attempt to change the trend, whatever it may be, in an effort to retain present-day customs. This had best be left to geniuses or fools.

Second, you can "play the game." If individualism is temporarily eclipsed in America, and preventive and therapeutic medicine are to be regimented along with public health administration, enter this new day with enthusiasm and a spirit of cooperation. If medicine must become bureaucratic, do your best to reach the top in the new order, where you can do your part in making state medicine the finest type of medicine. Do this with only one object in view, to uphold the spirit and tradition of the best in our profession, applying it to the new order. In this way will you achieve lasting and self-respecting success.

If, again God forbid, we return to the Dark Ages, your function should be that of preserving the advances made up to now, while awaiting the dawn of a new era. Your lives should then be comparable to those of the monks of the Middle Ages, the monasticism which kept knowledge alive, albeit feebly, until the new day. In short, the third alternative is that of cloistered existence in research and teaching in schools of higher education.

There is a fourth alternative. It has neither the spectacular appeal of the first, the active appeal of the second, nor the self-abnegation of the third. It is the program which will be followed by the rank and file of you, the life that has made medicine a glorious profession, and has made doctors beloved by their people. This consists in aiding the sick, advising the disturbed, encouraging the feeble, comforting the bereaved, building health and happiness for the individual, and letting the more ambitious worry about the first three alternatives.

Physicians are, on the whole, more highly evolved ethically than many other groups. Furthermore, the doctor is in less conflict with current politico-social trends than are others. The future will bring fewer basic changes in medicine than in other fields.

Each of us will find his function in one or another of these categories, and each will be doing his part to preserve the human race from deterioration. We are fortunate in that none of the schedules calls for wanton destruction of our fellow men, although this is mandatory for others, if humanity is to persevere. Nevertheless, we should be willing to fight, with arms if necessary, for that which we know to be best for the human race. Today, Hell hereafter disturbs us not at all. But Hell on earth is at our doorstep. Let us all become members of the new religion of science, the religion of moral and intellectual decency; a religion which is so jealous of human progress that it is willing to destroy ruthlessly all deterrents; a religion of ethics, whose decalogue may some day contain no prohibitions—no "Thou shalt nots"—but whose code of human relationships is summarized in the English phrase, "Is it cricket?"

It would seem appropriate in closing to read you a few words from an essay on "The Evolution of the Superman," written many years ago by Dean Victor C. Vaughan, who was beloved by all who knew him during his forty years' service with this School.

"Men are mortal but man is immortal. The individual lives are at best but a span, but the race continues. Numberless finites make up the infinite, and yet the soundness of the part determines the perfection of the whole."

It rests, then, with us as individuals, each to do his part, great or small, in assuring to posterity the continued upward progress which ethical man has striven so laboriously to deserve.

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CLINICAL AND EXPERIMENTAL

PHARMACOLOGY OF THYMOXYETHYLDIETHYLAMINE*

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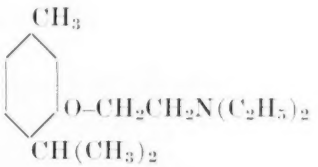
THYMOXYETHYLDIETHYLAMINE is a phenolic ether with the following chemical structure:



The free base is an oil, insoluble in water. The readily formed hydrochloride dissolves in water, a 3 per cent solution having a pH 3.0. Such solutions are unstable, primarily because of photosensitivity of the compound; however, they may be kept intact for a period of months in sealed ampoules under nitrogen and stored in the dark.

This compound was first described by Anan[1] who called attention to its sympathicolytic properties resembling those of the ergot alkaloids. Asakura[2] showed that it could inhibit the appearance of the adrenalin-induced hyperglycemia in the intact animal. Bovet and Maderni[3] showed that thymoxyethyldiethylamine, together with a number of other phenolic ethers, was capable of producing an ergotoxin-like reversal of the pressor effect of epinephrine on anesthetized dogs; this action they attributed to a paralysis of the sympathetic end plates. Levy and Ditz[4] made a similar observation on thymoxyethyldiethylamine and a number of its substitution compounds, describing the action as similar to that of ergotoxin or yohimbine in its ability to cause a reversal of the usual epinephrine effects.

Okazaki[5, 6] has given a detailed description of the pharmacologic properties of the methyl homologue of this compound, thymoxyethyldimethylamine. He points out that the compound has an effect as potent as that of ergotamine on the isolated rabbit uterus, that it is considerably less toxic than ergotamine (frogs), and that it is capable of causing a reversal of the typical adrenalin pressor effect. According to Staub,[7] this compound is in use in Japan as a therapeutic agent under the name "Tostramin."

Another closely allied substance, the diethylamine ethyl ether of 2-methoxy-6-allyl-phenol, has been described by Eicholtz,[8] and by Schmidt and

*From the Research Laboratories of the Winthrop Chemical Company, Inc., Rensselaer.
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Scholl,[9] under the proprietary name "Gravitol," and has been used clinically as an oxytocic agent in the treatment of uterine inertia, etc.

Fourneau and his co-workers[10-12] pointed out that certain phenolic ethers, particularly thymoxyethyldiethylamine, have the capacity of counteracting the effects of histamine, both in vitro and in vivo. The typical histamine-induced contractions of isolated smooth muscle could be inhibited by the previous addition of this compound to the bath fluid, and guinea pigs could be protected from the parenteral administration of two lethal doses of histamine by the previous subcutaneous administration of doses of 40 mg. per kilogram of the thymol derivative. Similar doses could also save sensitized guinea pigs from lethal doses of horse serum which produced fatal anaphylactic shock in unprotected animals.[13] This protection was achieved without influencing the sensitizing-desensitizing mechanism, for such animals behaved in the same way as controls when a subsequent precipitating dose of horse serum was administered some days after the administration of the thymoxyethyldiethylamine.

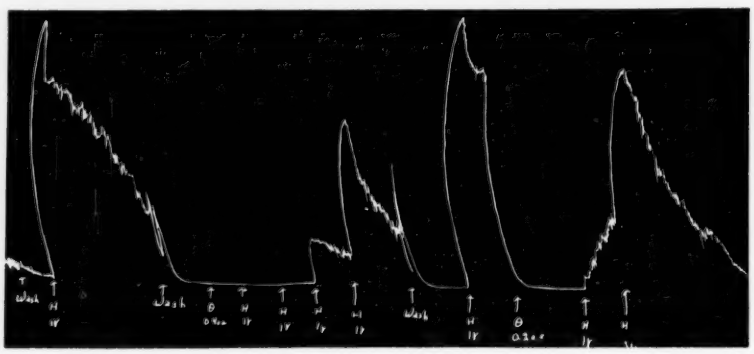

Fig. 1.—Loop of guinea pig jejunum in Webster's solution containing atropine. The normal response to the addition of 1γ of histamine to the bath fluid, followed by a wash and the addition of 0.4 c.c. of a 0.01 per cent solution of thymoxyethyldiethylamine. The normal histamine response is only observed after 4γ of histamine have been added. The preparation is washed, and the response to 1γ of histamine is again obtained. The addition of 0.2 c.c. of thymoxyethyldiethylamine causes an immediate relaxation of the contraction inaugurated by the histamine, and another normal histamine response is evoked after the addition of 2γ to the bath.

More recently, Rosenthal and Minard,[14] and Lambert and Rosenthal,[15] have described thymoxyethyldiethylamine as having a specific protective action against histamine and have used this observation to support their thesis that it is the local liberation of histamine which gives rise to the sensation of cutaneous pain. They have described thymoxyethyldiethylamine as possessing a local anesthetic action of about twice the potency of novocaine, but have failed to mention the intense local reactions resulting from the subcutaneous administration of this substance. We have repeatedly observed local edema, local necrosis, and sloughs following the subcutaneous administration of 1 per cent solutions. Staub has also called attention to this local effect and has pointed out that one drop of a 1 per cent solution will cause persistent congestion and lacrimation when placed on the rabbit's conjunctiva, and that urticarial papules may be produced when the material is passed through the skin by iontophoresis.

Staub has shown that thymoxyethyldiethylamine is capable of inhibiting the response of the isolated loop of guinea pig jejunum to doses of histamine

which would ordinarily induce maximal contractions. We have also observed this effect and have found that a quantitative relationship exists between the amount of histamine inhibited and the amount of thymoxyethyldiethylamine present. Thus, as illustrated in Fig. 1, doubling the amount of thymoxyethyldiethylamine added to the bath doubles the amount of histamine antagonized. Staub has shown that thymoxyethyldiethylamine not only inhibits the oxytocic effect of histamine but also causes almost complete inhibition of the normal response of acetylcholine and a suppression of the normal response to barium chloride. Rosenthal and Minard, however, claim that the antagonistic action of thymoxyethyldiethylamine for histamine is specific, and that small doses, capable of causing complete suppression of the isolated preparation's response

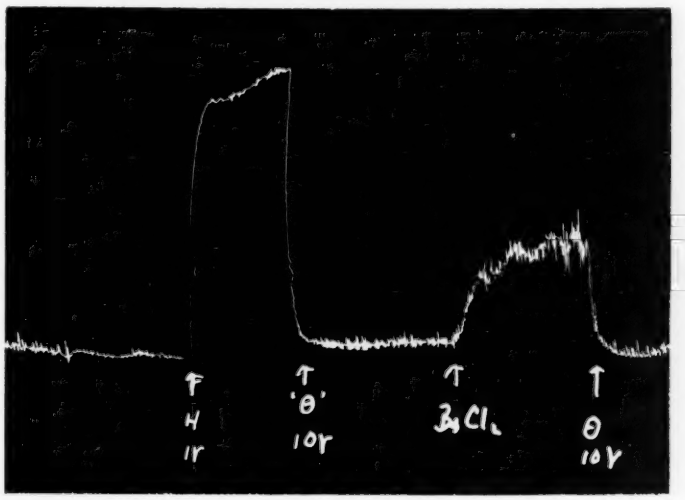

Fig. 2.—Loop of guinea pig jejunum in Webster's solution containing atropine. A maximal response is elicited by the addition of 1γ of histamine to the bath. The addition of 0.1 c.c. of a 0.01 per cent solution of thymoxyethyldiethylamine causes immediate relaxation of the muscle. The preparation is washed, and a second contraction is initiated by the addition of 20 mg. of barium chloride. Relaxation occurs immediately when the same quantity of thymoxyethyldiethylamine is added to the bath.

to histamine, have little or no effect on the contractions resulting from the addition of barium chloride, acetylcholine, sodium sulfocyanide, potassium chloride, or tyramine to the bath fluid. Our results do not bear this out, but are in closer agreement with those of Staub. We have found that quantities of the thymol derivative which will cause suppression of histamine contractions of the isolated gut will also render that preparation incapable of responding to barium chloride, and that concentrations which will cause immediate relaxation of smooth muscle after histamine stimulation will have a similar effect on contractions resulting from the administration of barium chloride. This observation is not compatible with any claim for specific antihistamine action. Sufficiently high concentrations of most spasmolytic or narcotic agents will curtail or completely inhibit the responsiveness of smooth muscle to oxytocins. Katz[16] has pointed out the fact that chloral hydrate, urethane, and ether will inhibit the response of smooth muscle to histamine. Downs and one of us (D. R. C.)[17] have shown that dibromoethylene behaves similarly with respect to histamine and the ergot alkaloids, but does not inhibit the contractions in-

itiated by the addition of pituitrin. This does not mean that these substances have a specific antihistamine effect, but rather that the muscle fibers are narcotized, or rendered incapable of responding to oxytocic stimuli. We are of the opinion that this is equally true of thymoxyethyldiethylamine.

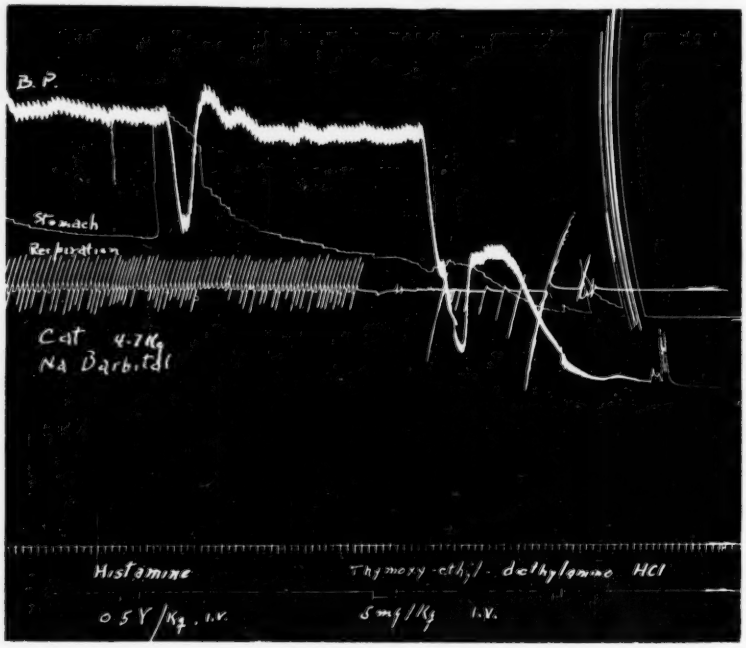

Fig. 3.—Cat under sodium barbital anesthesia. Record of carotid blood pressure, gastric contractions, and respirations. Time in five-second intervals. The fall in the blood pressure and the contraction of the smooth muscle resulting from the administration of 0.5γ of histamine per kilogram are first recorded. This is followed by the intravenous administration of thymoxyethyldiethylamine (5 mg. per kilogram) which causes the death of the animal.

The parenteral administration of thymoxyethyldiethylamine to intact animals gives rise to a number of dramatic effects. To begin with, the compound is quite toxic. Staub gives the toxicity in Table I. She points out that toxic

TABLE I

ANIMAL	M. L. D. (MG./KG.)	
	SUBCUTANEOUS	INTRAVENOUS
Guinea pig	200	20
Mouse	500	25
Rabbit	200	25

symptoms occur after the administration of doses as small as 10 per cent of the minimal lethal dose. We have observed fatal outcomes from the administration of doses as small as 4 mg. per kilogram intravenously to cats under sodium barbital anesthesia. The first effect is a slowing of the respiratory rhythm associated with a diminution in the amplitude of the respiratory movements. This is followed immediately by a precipitous fall in the blood pressure which is maintained at the shock level for about one minute. During this time apnea sets in. When the apnea has become complete, the heart fails and the blood pressure falls to zero.

It should be pointed out that the vasomotor system is rendered incapable of responding to potent stimulation during the time the blood pressure is at the shock level, for the administration of epinephrine in doses as large as 0.5 c.c. of a 1:10,000 dilution failed to evoke a significant response.

The intravenous lethal dose was determined for adult male rats, weighing between 350 and 400 Gm. The results, which are shown in Table II, are of the same order as those described by Staub.

TABLE II

NUMBER OF ANIMALS	DOSE (MG./KG.)	RESULTS		
		RECOVERED	DIED	NOTES
2	3.0	2	0	Nil
2	6.0	2	0	Nil
2	7.5	2	0	Nil
4	9.0	4	0	Convulsive movements during and immediately after administration. Rapid recovery
2	12.0	2	0	As above
2	13.5	2	0	As above. Marked dyspnea
2	15.0	2	0	As above. Symptoms more marked
5	16.5	3	2	Deaths from 0.5 to 5 minutes after administration Dyspnea and convulsions
10	18.0	2	8	As above
3	19.5	0	3	As above

Subcutaneous toxicity was determined on mice, and here again our figures agree with those obtained by Staub. Doses as small as 25 mg. per kilogram, however, produced marked inflammatory reactions at the sites of injection. When the dose level was increased to 100 mg. per kilogram, the inflammatory reaction was intense; the skin and subcutaneous tissues first became erythematous and edematous, the skin underwent necrosis, and the involved tissues sloughed away. Table III shows the results of the subcutaneous administration of an aqueous solution (1 per cent) of thymoxyethyldiethylamine hydrochloride to mice.

TABLE III

NUMBER OF ANIMALS	DOSE (MG./KG.)	RESULTS		
		RECOVERED	DIED	NOTES
5	200	5	0	Apathy and dyspnea followed by convulsive twitchings. Sloughs at injection sites
5	250	5	0	As above
5	300	5	0	As above. Symptoms more marked
5	350	2	3	As above. Deaths 24 to 48 hours after administration
5	400	1	4	As above

Henry,[18] working with Staub, attempted a clinical trial with this material, but found that man did not tolerate it. Within one and one-half hours after the oral administration of doses as small as 1.5 mg. patients complained of a sequence of disturbing side reactions. The voluntary muscles became stiff and sore, those muscles which had been fatigued by previous exercise being the first to be involved. This was followed by dyspnea and dysphagia, hyperesthesia of the skin, and lowered blood pressure. In one individual the dyspnea was

sufficiently marked to cause a feeling of suffocation of sufficient intensity as to give rise to considerable anxiety. In this patient the toxic reactions persisted for three hours. Increasing the dose caused an exacerbation of the symptoms previously described. When the level reached 20 mg., the muscle "stiffness" became acute muscular pain, the dyspnea was intensified, and nausea and vomiting occurred. The patients suffered from such intense headache that they were unable to maintain an erect posture.

Staub showed that guinea pigs may be protected from two lethal doses of parenterally administered histamine if a previous subcutaneous injection of 40 mg. per kilogram of thymoxyethyldiethylamine had been administered. We have been able to verify this observation.

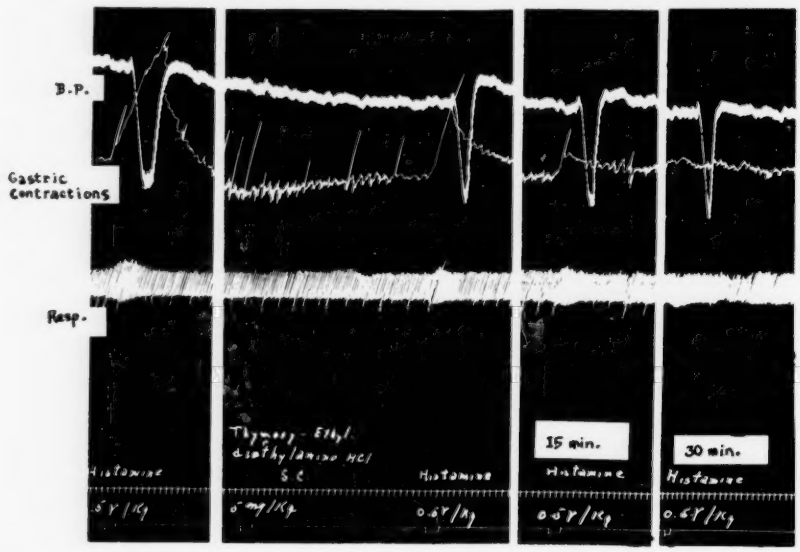

Fig. 4.—Cat under sodium barbital anesthesia. Record of carotid blood pressure, gastric contractions, and respiration. Time in five-second intervals. The fall in blood pressure and the contraction of the smooth muscle of the gut resulting from the administration of 0.5γ per kilogram of histamine is first recorded. This is followed by the subcutaneous administration of 5 mg. per kilogram of thymoxyethyldiethylamine. The fall in the blood pressure resulting from the subsequent administration of the same dose of histamine is not affected.

The thymol derivative has no effect on histamine in vitro; that is, it will not combine with histamine to produce an inactive compound, nor will it convert it to an inert form. This is demonstrated by the following experiment:

Fifty milligrams of thymoxyethyldiethylamine were added to 100 c.c. of a phosphate buffer solution at pH 7.4, containing 1 mg. of histamine monohydrochloride. This system was thoroughly oxygenated and incubated at 37° C. for twenty-four hours, during which time it was under continuous agitation. At the end of this time the solution produced characteristic vasomotor responses in the intact cat, which differed in no way from those evoked by the administration of an equal quantity of untreated histamine. Under the same conditions, one unit of histaminase (Torantil) will inactivate 1 mg. of histamine monohydrochloride.

When the compound is administered subcutaneously to the intact anesthetized animal, a number of reactions occur which are dependent on the size

of the dose. Doses up to 10 mg. per kilogram produce no apparent effect. There is a slight fall in the blood pressure, but there is no effect on the respiratory rate or rhythm. The lowering of the blood pressure by small doses of histamine is not altered by this order of dose.

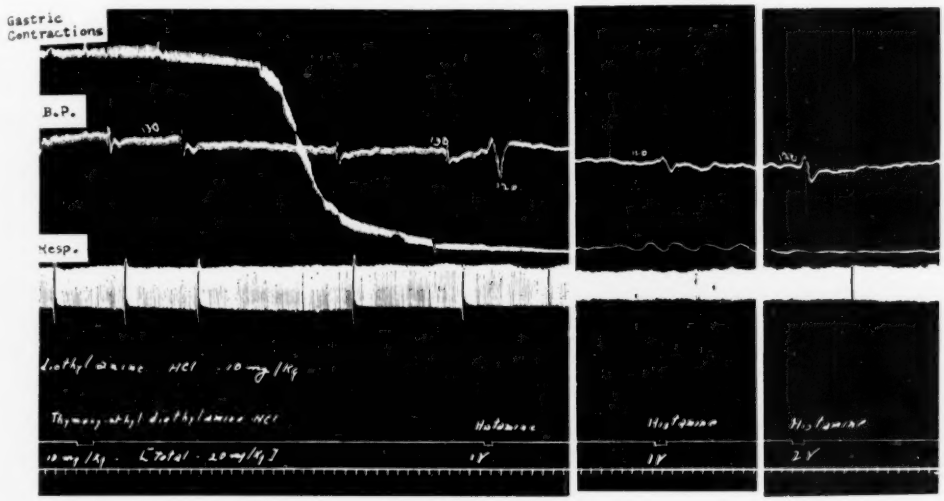

Fig. 5.—Cat under sodium barbital anesthesia. Record of gastric contractions, carotid blood pressure, and respirations. Time in five-second intervals. The administration of 20 mg. per kilogram of thymoxyethyldiethylamine causes a profound loss of tonus in the smooth muscle of the gut. The vasomotor reaction to 1γ of histamine is markedly diminished, but not completely abolished.

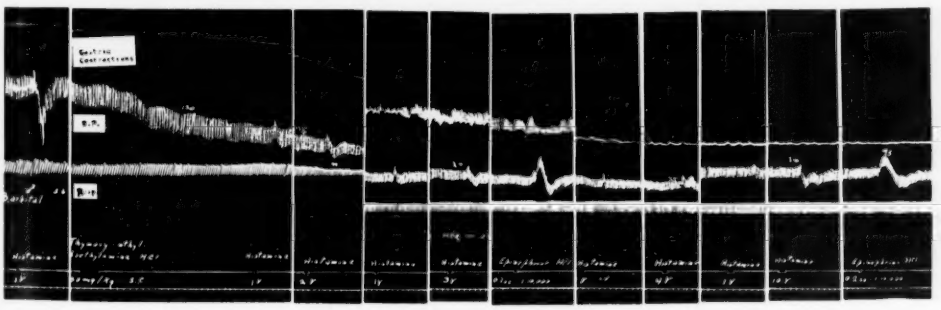

Fig. 6.—Cat under sodium barbital anesthesia. Record of gastric contractions, carotid blood pressure, and respiration. Time in five-second intervals. The normal response to 1γ of histamine is first recorded. This is followed by the subcutaneous administration of thymoxyethyldiethylamine (30 mg. per kilogram) which causes a definite fall in the blood pressure. Within three minutes the effect of 1γ of histamine has been abolished. One hour later the administration of 0.1 c.c. of a 1:10,000 solution of epinephrine produced no significant elevation of the blood pressure. Two hours after the administration of the thymoxyethyldiethylamine, 10γ of histamine produced no significant fall in the blood pressure, and 0.2 c.c. of epinephrine caused no rise in the blood pressure.

Increasing the dose to 20 mg. per kilogram causes a more marked fall in the systemic blood pressure which is associated with a slight increase in the respiratory rate. The most prominent effect of doses of this order is the marked loss in tonus of the smooth muscle. At this time the reaction to standardized doses of histamine is markedly reduced, but not completely abolished.

If the dose of thymoxyethyldiethylamine is increased to 30 mg. per kilogram, the fall in the blood pressure is more marked and the effects of small doses

of histamine (1 to 5 γ) are almost completely obliterated. This applies not only to the fall in the systemic blood pressure normally associated with the administration of such doses of histamine, but also to its stimulating action on smooth muscle.

It should be pointed out that at this time the peripheral paralysis of the vasomotor system is so complete that it becomes impossible to evoke a normal epinephrine response.

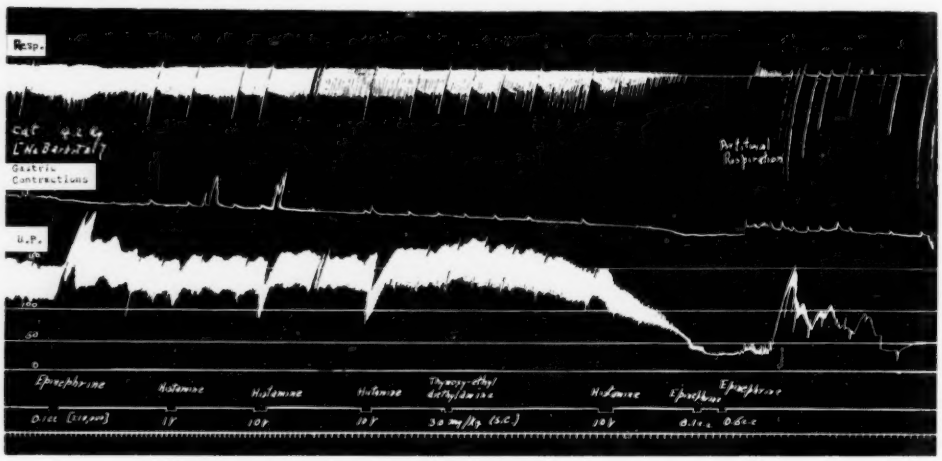

Fig. 7.—Cat under sodium barbital anesthesia. Record of respiratory movements, gastric contractions, and carotid blood pressure. (Blood pressure recorded on membrane manometer.) Time in five-second intervals. Normal responses to epinephrine and histamine are followed by the subcutaneous administration of thymoxyethyldiethylamine (30 mg. per kilogram). This causes a progressive fall in the blood pressure and results in death. There is no response to 0.1 c.c. of epinephrine (1:10,000), and a delayed, feeble response to 0.5 c.c. of epinephrine.

We have described characteristic responses to thymoxyethyldiethylamine, but these do not invariably result from the administration of this compound. We have at times seen fatal responses resulting from the subcutaneous administration of doses of this order which have not been associated with any modification of the usual histamine action on the vasomotor system.

SUMMARY

1. Thymoxyethyldiethylamine can inhibit the oxytocic effect of histamine in vitro. This action is not specific for histamine, for it will also inhibit the oxytocic effect of barium chloride.

2. Thymoxyethyldiethylamine can inhibit the fall in blood pressure in the cat resulting from the intravenous administration of histamine. Doses which are capable of producing this reaction cause a fall in the blood pressure and produce an ergotoxine-like reversal of the pressor effect of epinephrine.

3. Thymoxyethyldiethylamine can protect guinea pigs from the parenteral administration of two lethal doses of histamine.

4. Thymoxyethyldiethylamine has no inactivating effect on histamine in vitro.

5. Thymoxyethyldiethylamine is very toxic and highly irritant.

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MENOPAUSE ARTHRALGIA*

With a Preliminary Report on the Use of Stilbestrol

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THE diagnosis of menopause arthralgia could well be summed up as:

> "A female, fat and forty-four
> Who frowns from feverish knees,
> She 'fans in church,' her arms are sore,
> For hot flashes and flushes that freeze."

Seriously, this group of patients, whose symptoms are probably more annoying than pathologic in the early phase, do many times lead to a painful crippling condition of the weight-bearing joints later, as is borne out by histories obtained from older women who date their symptoms back to around the age of the meno-

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pause. At the present time complete clinical remissions are expected in the average patient who has received the diagnosis of menopause arthralgia. Should one of these patients fail to respond to treatment, we are more apt to question our diagnosis than the efficacy of the present established treatment.

Although the syndrome of joint and muscle pain, which occasionally accompanies or follows the artificial or the natural menopause, has never been widely accepted as a definite pathologic entity, nor the mechanism by which it takes place clearly demonstrated, the results from treatment of this syndrome certainly warrant grouping this type of rheumatic disease for special treatment. Francis Hall,[1] in his monograph on menopause arthralgia, has most ably described this syndrome and reports gratifying results in treatment from the use of estrogenic materials. In discussing one of Hall's papers[2] on this subject, I[3, 4] suggested the use of 10 c.c. of autohemotherapy reinforced with 101 degrees of artificial fever for one hour, together with the estrogenic materials in the treatment of those patients who fail to respond to the estrogenic materials alone. My results from the use of estrin compounds compare favorably to those reported by Dr. Hall.

PLAN OF TREATMENT

In the series reported here the following plan of management was applied to those carefully selected patients classed as menopause arthralgia:

A. General Supportive Measures:

1. Diet: Low carbohydrate, high vitamin on those patients who are overweight; otherwise a normal, well-balanced diet.
2. Avoidance of traumatic factors; this involves a myriad of things, such as general and local rest, proper shoes, avoidance of occupational strains, posture, etc.
3. Avoidance of climatic factors, such as cold, dampness, drafts, wet hands and feet, exposure, etc.
4. Foci of infections; these to be left intact throughout the active treatment or while rheumatic symptoms are present.
5. Supplementary vitamins; these to be used on patients who present a clinical deficiency. In this series the use of the vitamin B complex was most frequently indicated, especially in the group seen at the Arthritic Clinic at the Oklahoma University Hospital and the School of Medicine. Several of this group were diagnosed as having clinical pellagra.
6. Correction of disturbed or abnormal systems present, such as metabolism, gastrointestinal function, emotional stress, posture, and other pertinent factors affecting the patient's normal health.
7. Relief of pain.

B. "Specific" Measures:

1. In this phase the patients were divided into groups:
   (1) Injectable estrogenic hormone used in 60 patients who received an average of 10,000 units twice weekly over an average period of from four to ten weeks.

(2) Estrogenic hormone given by mouth or by insertion into the vagina. Thirty-four patients received 0.12 mg. in capsule form daily for an average period of four weeks.

(3) Stilbestrol (dihydroxy alpha, beta diethylstilbene) by subcutaneous injections; 60 patients received an average dose of 0.25 mg. (7,500 international units) twice weekly for an average of from four to ten weeks.

(4) Stilbestrol by mouth: 30 patients received 1 mg. in tablet form averaging from every two to four days.

(5) Autohemotherapy: As mentioned, this measure was supplemented only in those patients in whom the pathology had reached an advanced degree or who had failed to respond to the estrin or stilbestrol therapy alone.

(6) Artificial fever: As with the autohemotherapy, this measure is employed in those patients who have failed to respond to the estrin hormone or stilbestrol alone.

C. Physical Therapy Measures:

The same principles were applied here as in the other rheumatic phenomena. As a rule, the menopause arthritis patients have a lowered basal metabolism, and it is desirable to use measures which have a stimulating effect on the general metabolism. The more commonly used modalities are listed:

1. Heat: Local and general application. Except in the acute cases heat is usually followed with a brief application of cold. Warm baths of from fifteen to thirty minutes followed with a brief cold shower; contrast baths, hot paraffin, short wave, and hot fomentations, were helpful.

D. Orthopedic Measures:

1. Splinting and rest of acute areas.
2. Support where indicated. It is necessary to pay close attention to this phase of the management. It involves such features as splints, shoes, braces, and corsets. The knee is possibly one of the most important joints in that it is most frequently involved; once the disease process is started, it leads to degeneration of the joint. In our experience, the application of a leather knee cage brace reinforced with metal supports on each side hinged at the knee, has been most useful.

RESULTS

The results in these cases are listed in Table I. Briefly, they can be summed up as follows:

1. Sixty patients received stilbestrol by injection. In this group 80 per cent have had complete remission, 15 per cent improved, and 5 per cent failed to respond.

2. Thirty patients received stilbestrol by mouth. In this group 77 per cent had complete remission, 10 per cent improved, and 13 per cent failed to respond.

3. Thirty patients who had been receiving estrogenic hormones were changed to stilbestrol therapy. The patients were not informed of the change. Of these, 82 per cent noticed no change, 15 per cent stated they were better, and 3 per cent had increased pain.

4. Sixty patients in the control group received estrogenic hormones by injection. Of these, 85 per cent had complete remission, 10 per cent were improved, and 8 per cent failed to improve.

5. Thirty-four patients received estrogenic materials by mouth. Of these, 30 per cent had remission, 35.1 per cent were improved, and 35 per cent failed to improve.

It is interesting to note that those patients who had remission of rheumatic symptoms also had remission of the other symptoms accompanying the menopause. It is also interesting to observe that whereas the estrogenic material given by mouth failed as a whole to control the symptoms, the stilbestrol by mouth was largely adequate. Experience proved, however, that when stilbestrol is given by mouth, it is better to use 0.5 mg. instead of 1 mg., and that the tablets should be enteric coated. In the average case, the patient responded better if the stilbestrol was given daily. These measures largely eliminated the major complication, which was nausea.

TABLE I

THERAPY USED	NUMBER OF PATIENTS	REMISSION	IMPROVED	UNIMPROVED	COMPLICATIONS OR SIDE ACTIONS
1. Stilbestrol by injection	60	48 patients or 80%	9 patients or 15%	3 patients or 5%	Nausea 17% Sore breasts 5% Vertigo 1.6%
2. Estrin by injection	60	49 patients or 82%	6 patients or 10%	5 patients or 8%	Nausea 2% Sore breasts 4% Vertigo none
3. Stilbestrol by mouth	30	23 patients or 77%	3 patients or 10%	6 patients or 13%	Nausea 10% Sore breasts 5% Vertigo 1.6%
4. Estrin by mouth	34	10 patients or 29.4%	12 patients or 35.3%	12 patients or 35.3%	None
5. Autohemotherapy with estrin	14	12 patients or 85%	1 patient or 12.5%	1 patient or 12.5%	
6. Combined autohemotherapy and fever	15	12 patients or 80%	1 patient or 6%	2 patients or 14%	

DISCUSSION

The stilbene derivatives have been under careful experimental observation for the past six years. In 1934 Cook, Dodds, Hewett, and Lawson[5] described the estrogenic activity of a series of phenanthrene and dibenzanthracene compounds. It was shown that these substances can replace estrone in every way. In 1938 Dodds and Lawson[6] demonstrated that dihydroxystilbene had definite estrogenic activity.

More investigations were continued and the potent substance, 4,4; dihydroxydiethylstilbene (diethylstilbene stilbestrol), was discovered.[7-10]

Extensive pharmacologic tests of stilbestrol have been carried out on experimental animals, and the following facts have been established:

1. Geschickter,[11] in 1939, in discussing mammary carcinoma in the rat with metastasis induced by estrogen, suggests that "the mammary cancers induced in the rats by the injection of estrogenic substances are due to the physiologic changes produced rather than to the cancerogenic nature of the chemicals used."

2. Selye,[12] in 1939, in reporting on the toxicity of stilbestrol, concluded: "Experiments on the mouse and the rat indicate that when given in very high doses both estrodiol and diethyl stilbestrol may exert toxic effects. In the mouse, death may occur under the influence of such estrogens."

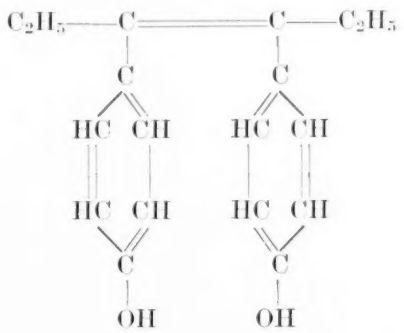
3. Loeser,[13] in 1939, in reporting on the clinical effect of diethylstilbestrol, states that this product has a marked substitution action in various forms of ovarian insufficiencies, but that 6 of the 15 patients in whom it was used had nausea or vomiting.

4. Others, MacGregor,[14] Bishop, Boycott, and Zuckerman,[15] Winterton,[16] Kellar and Sutherland,[17] Rees,[18] Karnaky,[19] Ehrhardt, Kramann, and Schäfer[20] in 1939 reported favorably on the use of stilbestrol. Karnaky[19] states that it is as potent when given by mouth as by injection and that no toxic effects were felt. Varanget,[21] reporting in the 1939 literature, however, advises against its use.

In the 1940 literature 33 papers were published from January to June. Of these, I was able to obtain 15. In these, 12 of the authors[22-33] advise the use of stilbestrol, while 3 of them[34-36] advise against its use. The universal objection is chiefly the side action of nausea and epigastric pain.

In our series 10 patients (17 per cent) complained of nausea. In all these, however, the drug was continued at a reduced dosage. In only one patient was it necessary to discontinue the drug. In using this preparation, however, all the factors contraindicating the use of the estrins must be closely observed and followed.

The terminology of stilbestrol is somewhat confusing due to the synonyms used. Diethylstilbestrol, 5,5, dihydroxydiethylstilbene, and diethylstilbene are used. The empirical formula is $C_{18}H_{20}O_2$. The structural formula:



It is a white, odorless, tasteless, nonhygroscopic powder. Less than 1 mg. will go into solution in 20 c.c. of water. It is soluble in most organic solvents and in vegetable oils. It is soluble in alkali hydroxides.

CONCLUSIONS

1. Forty-eight patients (80 per cent) diagnosed as having menopause arthralgia had satisfactory remissions following the injections of stilbestrol used in conjunction with other supportive measures.

2. Forty-nine patients (82 per cent) as controls responded satisfactorily to estrin injections along with the same supportive measures.

3. In 30 patients stilbestrol was successfully substituted for estrin with only 3 per cent having return of original symptoms.

4. Twenty-three patients (77 per cent) receiving stilbestrol by mouth had clinical remission as compared to 10 patients (30 per cent) who received estrin that responded by clinical remission.

If the foregoing findings are borne out by later investigators, and if complications not yet detected in the patient do not make their appearance, stilbestrol should supplant the use of the estrogenic hormone for two reasons: (1) It is effective when given orally. (2) The cost to the patient is greatly reduced. In considering the fact that the treatment is largely substitutional and must be continued for a comparatively long period of time, these two factors would justify the use of stilbestrol.

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605 N. W. 10TH STREET

CARDIAC DEPRESSION BY MERCURIAL DIURETICS*

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IN VIEW OF the fact that mercurial diuretics are often used in cases of edema and dropsy of cardiac origin, these experiments were performed on isolated turtle hearts to ascertain whether impaired cardiac action may follow their use in concentrations which prevail therapeutically, and to note the antidotal effect of sodium thiosulfate and certain cardiac stimulants.

A review of the abundant literature is not intended, but certain papers are cited for their pertinent interest. Salant and Kleitman[1] in 1922 studied the action of mercurial salts on the hearts of intact cats and dogs, and on the isolated hearts of frogs and turtles. Turtle hearts developed delirium cordis after twelve minutes of perfusion with mercuric chloride, one part in one million. This persisted for more than twenty minutes during subsequent perfusion with Ringer's solution. Similar effects were observed on the hearts of cats under urethane anesthesia, but of shorter duration. Wolf[2] in 1932, Molnar[3] in 1935, and Greenwald and Jacobson[4] in 1937, report cases of sudden death following the administration of mercurial diuretics to patients. Deaths following the

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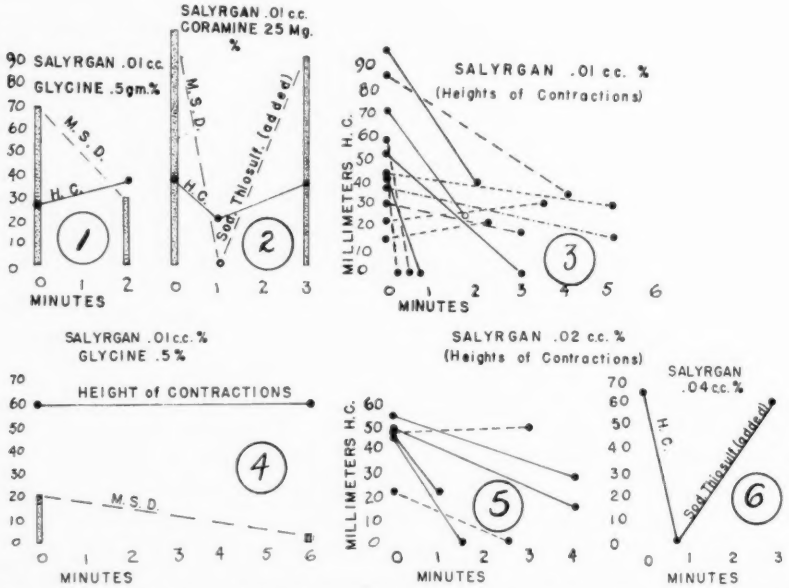
therapeutic administration of calomel are reported by Backer[5] in 1921, Burnet and Pestal[6] in 1924, and Póvoa[7] in 1927.

METHOD

Ringer's solution with the pH adjusted between 7.2 and 7.4 served as the vehicle of perfusion for all drugs used. The apparatus and technique were the same as used in previous publications.[8] Solutions of the mercurial diuretics, salyrgan and mercupurin, were prepared by diluting the 1 c.c. commercial ampoules. The most frequently used concentration in this study, 0.01 c.c. per 100 c.c. of Ringer's solution, had a mercury content of one part in 300,000.

RESULTS

In the charts the ordinates indicate by black dots the heights of cardiac contractions as written by the lever on the smoked drum, while the vertical columns indicate relatively the cardiac work done in terms of stroke distance per minute (M.S.D.); the figures in the abscissa indicate the perfusion time in minutes.



Figs. 1-6.—Fig. 1 shows height of contractions increased by glycine in the presence of salyrgan, 0.01 c.c. per 100 c.c. of Ringer's solution, but due to the slowed heart rate caused by salyrgan the cardiac work (M.S.D.) is decreased.

Fig. 2, both the height of contractions and the rate are decreased by salyrgan, 0.01 c.c. per 100 c.c., in the presence of coramine, but sodium thiosulfate causes immediate recovery.

Fig. 3, salyrgan, 0.01 c.c. per 100 c.c., decreased the height of contractions in 9 of 11 experiments.

Fig. 4, another experiment similar to Fig. 1.

Fig. 5, height of contractions decreased in 5 of 6 experiments by salyrgan, 0.02 c.c. per 100 c.c.

Fig. 6, salyrgan, 0.04 c.c. per 100 c.c., quickly brought height of contractions to zero; added sodium thiosulfate caused immediate recovery.

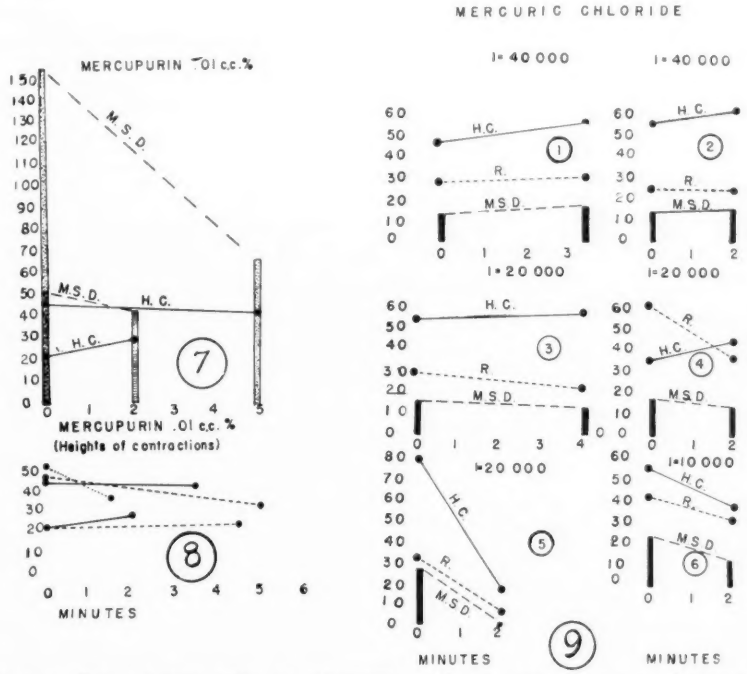
Salyrgan. Figs. 1 and 4 show that salyrgan perfused in a concentration of 0.01 c.c. per 100 c.c. in the presence of a cardiac stimulant (glycine 0.5 Gm. per cent) increased the height of contractions but decreased the cardiac work (M.S.D.).

Fig. 2 indicates that both the height of contractions and the M.S.D. are decreased by salyrgan, 0.01 c.c. per 100 c.c., in the presence of another cardiac stimulant (coramine 25 mg. per cent). Recovery is prompt with the addition of sodium thiosulfate, 10 mg. per cent, to the perfusate.

Fig. 3 shows 11 experiments wherein the same concentration of salyrgan was perfused. In nine of these, or 82 per cent, the height of contractions was decreased.

Fig. 5 shows five experiments wherein 0.02 c.c. per cent of salyrgan was perfused. In four of these, or 80 per cent, the height of contractions was decreased.

Fig. 6 shows the decrease in height of contractions caused by perfusion with salyrgan, 0.04 c.c. per cent, and prompt recovery when sodium thiosulfate was added.



Figs. 7-9.—Fig. 7 shows effect of mercupurin, 0.01 c.c. per 100 c.c. of Ringer's solution, on height of contractions and cardiac work (M.S.D.).
Fig. 8 shows heights of contractions in 5 experiments wherein mercupurin, 0.01 c.c. per 100 c.c. was perfused.
Fig. 9 shows graphs of 6 perfusion experiments with bichloride of mercury in concentrations of 1:40,000 to 1:10,000.

Mercupurin. Fig. 7 shows the results of mercupurin, 0.01 c.c. per cent, on the height of contractions and the minute stroke distance. In both experiments the amount of work done (M.S.D.) was decreased, but not to the same extent. In one the height of contractions was increased.

Fig. 8 shows the heights of contractions of five experiments on hearts perfused with the same concentration of mercupurin.

Mercuric Chloride. Fig. 9, graphs 1 to 6 inclusive, shows for comparison the effect of perfusion of the isolated turtle heart with bichloride of mercury in the concentrations of 1:40,000, 1:20,000, and 1:10,000 in Ringer's solution.

The concentration of 1:40,000 slightly increased the height of contractions and the rate with a consequent increase in the M.S.D. The susceptibility of the individual hearts varied, e.g., the concentration of 1:20,000, graph 5, in one heart was more depressing than that of 1:10,000, chart 6, in another heart.

Mercurous Chloride. Fig 10*A* is the second tracing of a heart tracing showing fully developed delirium cordis by perfusion with calomel in a concentration of 1:10,000, shaken and decanted, and the decanted fluid used as perfusate. When Ringer's solution was subsequently perfused, the irregularity persisted for several minutes, the mercury being difficult to wash out; but when sodium thiosulfate was perfused, the antidotal effect was immediate.

Fig. 10*B* is a specimen tracing from which the charts of Fig. 2 were derived. Notwithstanding the presence of coramine, 25 mg. per 100 c.c. with the mercurial diuretic, there was typical mercury depression evidenced by decrease in height of contractions followed by partial, then complete, heart block. Continuation of perfusion with the same solution plus sodium thiosulfate, 10 mg. per 100 c.c., resulted in immediate recovery. These tracings, 10*A* and 10*B*, are shown because they typically illustrate the cohesion of mercury salts in the cardiac tissues, and that sodium thiosulfate rapidly penetrates the tissues performing its well-known antidotal action.

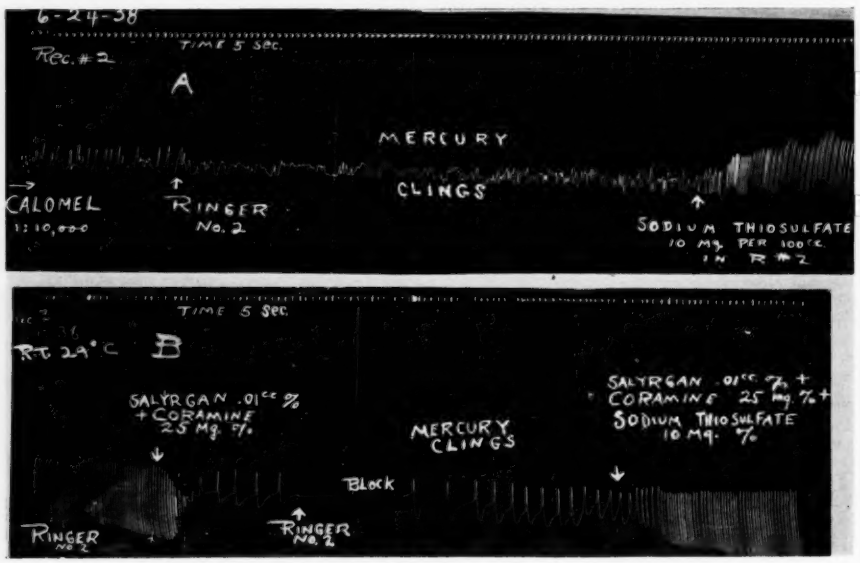


Fig. 10.—*A* (upper chart) shows the heart tracing after the full development of delirium cordis by perfusion with decanted Ringer's solution in which calomel 1:10,000 had been shaken. The mercury effect was not readily removed by perfusion with Ringer's solution until sodium thiosulfate was added; whereupon recovery was immediate. *B* (lower chart) is the experiment from which the graph in Fig. 2 was mathematically derived.

COMMENT

That the mercurial diuretics occupy a position of great usefulness in the treatment of selected cases of edema and dropsy cannot be denied. It is desired to emphasize that their effectiveness is due to their mercury content, and that their toxicity is not appreciably different from that of a similar concentration of mercury in other forms. No claim is made here that tolerance to mercury is

identical in the different species insofar as the cardiac tissues are concerned, nor is this research intended to discourage the proper use of the mercurial diuretics, under close medical supervision, in suitable cases.

CONCLUSIONS

A study was made of the toxic effects of mercurial diuretics and the protective effects of various heart stimulants on isolated turtle hearts.

1. The mercurial diuretics possess a toxicity to the heart with reference to their mercury content that is not essentially different from that of inorganic mercury compounds. This side action is apart from diuresis and may sometimes impede it.

2. The purine derivatives especially, and to some extent certain of the other cardiac stimulants, when used in conjunction with mercurial diuretics, somewhat decreased the toxicity to the heart when the dose of the latter was sufficiently small to be of low toxicity. These cardiac stimulants, however, had relatively little antidotal action.

3. Sodium thiosulfate perfused through the mercury-poisoned heart in complete block rapidly penetrates the tissues and restores the normal force and rhythm whether the toxic agent is one of the mercurial diuretics, or calomel, or bichloride of mercury. This antidotal action takes place even if the mercury compound is continuously perfused in the presence of the antidote.

Thanks are extended to Dr. Martin H. Fischer for constructive criticism and suggestions during the conduct of this research.

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THE EFFECT OF SUPPLEMENTAL AMYLASE ON DIGESTION*

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JUDGING from the comments in many textbooks of materia medica and therapeutics, it must be concluded that, at the present time, most authors question the value of enzyme therapy. This is probably due, at least in part, to a report by Bastedo.[1] He submitted a questionnaire pertaining to the value of digestive enzymes, to a representative group of gastroenterologists. On the basis of their replies, he was forced to conclude that at the time of his survey, these agents were of minor importance in therapeutics.

Two widely held conceptions appear to be largely responsible for the low esteem in which enzyme therapy is held. It is generally believed that (a) deficient digestion is rarely, if ever, a cause of symptoms, and that (b) in most cases enzyme therapy is necessarily doomed to failure because the administered enzyme would be quickly inactivated by the acid gastric juice. That both of these concepts are erroneous has been established conclusively. It has been shown repeatedly in laboratory animals in which pancreatic juice has been prevented from reaching the intestine that enzyme therapy exerts a potent effect on the quantity of food assimilated.[2] Silverman, Denis, and Leche[3] have shown that a number of different pancreas preparations augment the concentration of enzymes in the intestine, and Ivy, Schmidt, and Beazell[4] have shown that, when properly administered, approximately 85 per cent of ingested enzyme reaches the intestine in an active form. More recently, Beazell, Schmidt, and Ivy[2] have discussed the value of enzyme therapy in the treatment of achylia pancreatica. In 4 patients suffering from a complete suppression of the external secretion of the pancreas, they found that adequate enzyme therapy reduced the quantity of fat and nitrogen eliminated in the feces by an average of 63.3 per cent and 62 per cent, respectively. Further, the clinical response of the patients was gratifying in all cases.

The investigation herein discussed was undertaken to test further the potentialities of enzyme therapy. It was instituted to provide answers to two problems: (1) What is the effect of supplementary diastase on starch digestion in the stomach? (2) Is that fraction of the supplementary diastase which reaches the intestine in an active form capable of effective hydrolysis? The first of these two problems has already been attacked by Ivy, Schmidt, and Beazell.[4] They were primarily concerned with the effect of diastase on the rate of digestion, rather than on the total quantity of starch digested. Furthermore, in most of their studies they employed a cereal (farina) as a test meal, so their observations cannot be unequivocally used as a basis for anticipating

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the effect of supplementary diastase in the presence of a mixed meal. For these reasons, it appeared desirable to repeat and extend their experiments.

For a number of reasons taka-diastase* was chosen as the test enzyme preparation. First, it is a potent diastatic ferment having an activity of 0.96 Willstätter[5] and 11,260 Davison[6] units per gram; second, it is active over a relatively wide pH range; and third, in powder form it can be stored indefinitely without alteration in activity.

EFFECT OF TAKA-DIASTASE ON GASTRIC DIGESTION IN NORMAL HUMAN BEINGS

Methods.—The details of the experimental methods and calculations have been described and discussed in a previous communication.[7] Briefly, the experiment consisted of feeding normal young adult males a meal of known composition, withdrawing it from the stomach at the end of a specified period of time, and determining the ratio of "digested" to "undigested" carbohydrate and, in certain experiments, protein in the recovered material. The effect of the taka-diastase was determined by comparing the results of one or more control experiments with one or more in which taka-diastase was taken with the standard meal.

Twelve clinically normal young adult males were used as subjects for the experiment. In the initial phases a test meal consisting of 150 Gm. of beef steak, 150 Gm. of boiled potatoes, 50 Gm. of green peas, 10 Gm. of butter, and 200 c.c. of water was employed. When used, 2 Gm. of taka-diastase were dissolved in the water which was sipped throughout the meal. Five persons who were capable of voluntarily regurgitating the gastric contents for analysis were tested with this meal. During preliminary experiments it became apparent that under the conditions of the experiment taka-diastase effected no augmentation of gastric starch digestion. Apparently in these normal healthy human beings, the ingestion of an appetizing meal stimulated the flow of sufficient saliva to effect maximum digestion so that supplementary amylase had no demonstrable effect. Therefore, in order to determine whether taka-diastase was capable of hydrolyzing starch in the stomach, it was necessary to have the subjects voluntarily induce a salivary deficiency. This was accomplished by having them "bolt" the meal, and then during the hour the meal remained in the stomach, swallow as little saliva as possible.

Subsequently, this meal was abandoned in favor of a finely divided homogeneous meal for the following reasons: (a) Only a limited number of subjects were capable of cooperating in an experiment requiring regurgitation of the meal. (b) By resorting to a finely divided meal it was possible to evacuate completely the stomach by means of an Ewald tube, and to (c) determine the dilution of the meal by the gastric juice. This latter determination made it possible to calculate the fraction of the ingested taka-diastase remaining in the stomach in an active form at the termination of the experiment, and the fraction of the digested material recovered from the stomach that represented preformed digested material contributed by the meal.

*Product of Parke, Davis & Co.

The finely divided meal consisted of 100 Gm. of ground lean beef and 30 Gm. of potato starch boiled in sufficient water to yield a final volume of 400 c.c. Twenty milligrams of phenolsulfonephthalein was dissolved in the water used in preparing the meal. The dilution of the dye served to determine the dilution of the meal by the gastric juice and saliva. When this meal was employed, it was unnecessary to induce a state of artificial salivary deficiency in order to demonstrate augmentation with taka-diastase.

Results.—The results obtained with the unground meal are summarized in Table I. Of the 5 persons tested, 3 showed a decided increase in the quantity of starch digested in the stomach when taka-diastase was added to the meal, and the remaining 2 showed no change. The results are significant, since whenever a change occurred it was always in favor of more complete digestion when taka-diastase was taken with the meal.

TABLE I

EFFECT OF TAKA-DIASTASE ON GASTRIC DIGESTION OF THE STARCH IN A NORMAL MEAL

(Two grams of taka-diastase were used. Normal subjects voluntarily induced salivary deficiency.)

SUBJECT	COMPOSITION OF MEAL				MATERIAL RECOVERED FROM STOMACH						TOTAL SUGAR*		% DIGESTION	
	TOTAL CARBOHYDRATE		TOTAL SUGAR*		VOLUME		pH		TOTAL CARBOHYDRATE					
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
J. W.	44.8	52.4	8.4	13.9	85	110	3.9	3.9	4.08	2.86	1.73	1.76	23.7	36.4
L. R.	47.6	49.6	10.1	12.1	100	125	4.2	4.8	3.40	4.00	2.02	2.50	38.3	38.2
T. K.	36.4	42.6	6.8	7.9	155	125	4.0	3.6	5.78	5.05	2.32	2.75	21.5	35.1
H. A.	44.8	52.4	8.4	13.9	385	270	3.7	4.0	21.60	14.60	8.17	6.80	18.9	20.0
J. C.	36.4	42.6	6.8	7.9	225	325	4.1	3.5	8.10	9.56	4.41	7.15	35.7	55.5
Average													27.6	36.7

*As maltose.

The results obtained with the finely divided meal are summarized in Tables II and III. Taka-diastase had no effect on the digestion of protein, which is not surprising since it possesses only feeble proteolytic activity.

In the case of starch digestion, on the other hand, taka-diastase effected a significant augmentation in all persons with a 2 Gm. dose, and in at least 5 of the 8 persons with a 1 Gm. dose. In the cases in which duplicate control determinations were conducted, satisfactory checks were obtained in all persons except one. This one was also erratic in his response to taka-diastase, a greater effect being obtained with 1 Gm. than with 2 Gm. It should be noted, however, that in the presence of taka-diastase digestion was more complete than in either of the control experiments.

In no case did a significant fraction of the taka-diastase remaining in the stomach retain its activity.

EFFECT OF TAKA-DIASTASE ON GASTRIC DIGESTION IN THE DOG

Having established that conditions in the human stomach are not incompatible with effective hydrolysis by taka-diastase, it became desirable to study its action under circumstances which made it possible to determine total gastric

TABLE II

EFFECT OF TAKA-DIASTASE ON THE GASTRIC DIGESTION OF STARCH IN THE NORMAL HUMAN SUBJECT

(Finely divided test meal)

SUBJECT	VOLUME RECOVERED FROM STOMACH			RECOVERED VOLUME CORRECTED FOR DILUTION			pH RECOVERED MATERIAL			SUGAR* RECOVERED FROM STOMACH (AS MALTOSE)			STARCH† EQUIVALENT OF RECOVERED SUGAR			CALCULATED STARCH CONTENT OF RECOVERED MATERIAL			PER CENT OF STARCH DIGESTED TO MALTOSE		
	C	T_2	T_1	C	T_2	T_1	C	T_2	T_1	C	T_2	T_1	C	T_2	T_1	C	T_2	T_1	C	T_2	T_1
H. W.+	380	425	450	324	304	338	2.2	3.7	2.2	4.9	6.9	4.7	4.5	5.5	4.4	24.3	22.8	25.4	18.5	24.1	17.4
J. Wa.	250	310	350	227	282	292	1.7	2.7	2.8	3.0	7.8	4.1	2.8	6.2	3.9	17.0	21.2	21.9	16.5	29.2	17.6
R. M.+	285	290	205	170	236	143	2.6	2.5	3.3	2.3	4.9	2.7	2.1	3.9	2.5	12.8	17.7	10.7	16.4	22.0	23.6
C. J.	245	285		188	235		1.9	2.8		3.7	6.4		3.4	5.9		14.1	17.6		24.1	33.5	
W. L.+	330	400	340	207	333	263	2.5	2.9	2.6	4.0	7.6	6.1	3.7	7.0	5.7	15.5	25.0	19.7	24.0	28.0	30.8
M. B.+	109	160	135	99	133	123	2.4	2.6	2.8	2.1	3.6	3.0	1.9	3.4	2.8	7.4	9.9	9.2	25.7	34.4	33.0
J. W.+	185	145	125	163	118	99	2.6	3.3	3.3	3.4	3.8	2.0	3.1	3.6	1.9	12.2	8.9	7.4	25.4	40.5	27.5
G. L.+	257	350	310	229	239	246	1.5	1.8	5.4	4.3	8.1	9.5	4.0	7.5	8.9	17.1	17.9	18.4	22.4	41.8	48.2
V. S.	215		260	179		142	1.7		1.8	2.4		4.0	2.2		3.7	13.4		10.7	16.4		34.7
Average	250	296	272	198	235	206	2.1	2.8	3.0	3.3	6.1	4.5	3.0	5.40	4.23	14.8	17.6	15.4	20.2	30.7	27.5

*Corrected for preformed sugar from meal and taka-diastase.

†Sugar × 0.925.

+Control values represent average of two determinations.

TABLE III

EFFECT OF TAKA-DIASTASE ON GASTRIC DIGESTION OF PROTEIN BY NORMAL HUMAN SUBJECTS

(Finely divided meal)

SUBJECT	VOLUME RECOVERED FROM STOMACH		RECOVERED VOLUME CORRECTED FOR DILUTION		pH RECOVERED MATERIAL		TOTAL NITROGEN INGESTED		TOTAL SUBPROTEOSE NITROGEN INGESTED		TOTAL NITROGEN RECOVERED		TOTAL SUBPROTEOSE NITROGEN RECOVERED		PER CENT OF INGESTED NITROGEN EVACUATED FROM STOMACH		% DIGESTION OF NITROGEN REMAINING IN STOMACH*	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
J. W.	230	125	197	99	2.6	3.3	3.04	3.41	0.336	0.304	1.23	0.470	0.202	0.085	59.5	86.0	3.0	2.10
H. W.	440	450	366	338	1.8	2.2	2.45	3.41	0.336	0.304	1.63	1.69	0.317	0.292	33.4	50.5	0.55	2.10
R. M.	230	205	144	143	3.5	3.3	2.98	2.63	0.312	0.304	0.930	0.738	0.076	0.096	69.0	72.0	0	0
J. Wa.	215	350	140	292	1.8	2.8	2.98	2.45	0.312	0.336	0.615	1.79	0.093	0.315	79.4	27.0	0	3.90
G. D.	270	310	211	246	1.4	5.4	2.63	2.43	0.304	0.336	0.698	0.837	0.173	0.285	73.5	71.8	1.85	9.45
V. S.	215	260	179	142	1.7	1.8	2.63	2.43	0.304	0.336	0.636	0.518	0.138	0.148	75.8	78.8	0.31	5.80
W. L.	340	340	188	263	2.5	2.6	2.39	2.77	0.304	0.344	0.71	1.410	0.190	0.306	70.0	49.0	6.6	5.7
M. B.	58	135	38	123	2.1	2.8	2.39	2.77	0.304	0.344	0.194	0.443	0.042	0.124	92.0	86.0	6.7	4.1
Average	250	272	183	206	2.18	3.02	2.69	2.78	0.314	0.326	0.831	0.988	0.154	0.206	69.0	65.0	2.4	4.2

*Corrected for preformed soluble nitrogen contributed by meat.

digestion. In the studies on the human subjects only the state of digestion at the end of a specified period of time could be determined. In order to determine total digestion in the stomach, a duodenostomy or duodenal fistula must be present so that the total gastric discharge can be collected for analysis as it is evacuated through the pylorus. Therefore, in 3 dogs the bile was diverted to the lower jejunum by means of a cholecystojejunostomy, the pancreatic juice was prevented from reaching the intestine by resecting the portion adherent to the duodenum, and a duodenostomy was created. By introducing a large catheter (28 F) into the duodenum through the fistula, it was possible to collect the uncontaminated gastric contents as they were evacuated through the pylorus. Since the dog's saliva contains no ptyalin, results in the dog might, with justification, be used to predict what would occur in the ptyalin-deficient patient.

Methods.—The meal used in these experiments was identical to the finely divided meal used in the experiments on human beings. In the case of one animal it was necessary to dilute the meal and administer it by means of a stomach tube. In the experiments in which taka-diastase was tested, 2 Gm. were mixed with the meal immediately before administration. Continuous collection of the discharge from the fistula was instituted immediately after the meal was ingested and continued until the stomach was empty. The material was permitted to accumulate in the collecting receptacle for periods of thirty minutes before being sampled for analysis. The analytical methods have been described and discussed previously.[7]

Results.—The results of this experiment are summarized in Table IV. In the case of each animal the results represent an average of three control determinations and three determinations with taka-diastase. It is unnecessary to record the results of individual experiments, since the variations were inconsequential. These results reveal the following facts: (1) The fraction of the ingested starch digested in the stomach was markedly augmented by taka-diastase in all cases. The average for the 3 animals was 7.58 per cent digestion without taka-diastase, and 38.9 per cent digestion with taka-diastase. These values demonstrate that in the absence of ptyalin, starch digestion in the stomach is of no significance, and that adequate substitution therapy will reduce a large fraction of the starch to simple sugars. (2) The fraction of ingested taka-diastase reaching the intestine in an active form is subject to wide individual variation, being dependent on the rate of secretion of acid by the stomach. In animal No. 2, in which the fall in pH approaches that in man, the diastase was almost entirely inactivated in the stomach. It must be concluded that when administered in this manner the effect of taka-diastase is confined almost entirely to the period during which it remains in the stomach. (3) The addition of taka-diastase to the test meal had no significant effect on the rate of emptying of the stomach.

EFFECT OF TAKA-DIASTASE ON STARCH DIGESTION IN THE SMALL INTESTINE

Having demonstrated that taka-diastase has a positive and significant effect on starch digestion in the stomach, the effect of that fraction that escapes inactivation by the gastric juice was investigated.

TABLE IV

DOG	VOLUME OF FLUID RECOVERED	RECOVERED VOLUME CORRECTED FOR DILUTION	TIME (HR.)	ACID			SUGAR RECOVERED (AS MALTOSE)	STARCH EQUIVALENT OF RECOVERED SUGAR	CALCULATED STARCH OF RECOVERED FLUID	PER CENT OF INGESTED STARCH DIGESTED IN STOMACH	DIASTASE RECOVERED (MG. OF TAKA*)	% OF INGESTED TAKA EVACUATED IN ACTIVE FORM
				FREE	TOTAL	pH						
1												
Control	169	115	½	0	44	5.1	0.45	0.42	8.6	4.85	0	
			1	0	44	4.4						
			1½	0	52	3.3						
			2	0	52	3.5						
Taka-diastase (2 Gm.)	198	165	½	0	26	5.1	6.59	6.15	12.4	49.5	513	25.6
			1	0	37	4.8						
			1½	0	47	4.3						
			2	0	57	3.8						
2												
Control	442	356	½	0	40	4.2	1.7	1.6	26.7	6.0	0	
			1	0	59	3.8						
			1½	10	64	3.4						
			2	20	72	3.1						
			2½	25	75	2.6						
			3	25	80	2.2						
Taka-diastase (2 Gm.)	321	307	½	2	40	4.0	8.8	8.2	23.0	35.6	35	1.7
			1	2	52	3.6						
			1½	7	56	2.8						
			2	10	68	2.6						
			2½	20	73	2.2						
			3	27	85	2.2						
3												
Control	73	69	3	2	40	3.6	0.66	0.62	5.2	11.9	0	
Taka-diastase (1 Gm.)	176	165	1½	0	32	3.6	4.20	3.9	12.4	31.7	441	44.1

*Corrected.

Methods.—Dogs were anesthetized with pentobarbital, the abdomen was entered through a midline incision, and a sigment of intestine from 70 to 90 cm. long was isolated and cannulated at both ends. The segment was washed first with 500 c.c. of water, then 500 c.c. of tenth-normal hydrochloric acid, and then another 500 c.c. of water. From 70 to 100 c.c. of either 3 or 4 per cent potato starch paste was then introduced and permitted to remain for one hour. The unabsorbed fraction of the paste was permitted to drain out, after which the segment was flushed out with 100 c.c. of water, which was collected and combined with the residual paste that had been collected. The segment was then washed with an additional 500 c.c. of water, and the experiment was repeated with 500 mg. of taka-diastase added to the starch paste immediately before introduction. The recovered samples were assayed for amylase, and the reducing sugar and the pH were determined.

TABLE V

EFFECT OF TAKA-DIASTASE ON STARCH DIGESTION IN THE INTESTINE

DOG		CONC. STARCH PASTE (%)	VOL. STARCH PASTE INTRODUCED (C.C.)	VOL. WASH FLUID	TOTAL VOLUME RECOVERED	SUGAR RECOVERED* (GM.)	STARCH EQUIVALENT OF SUGAR	% STARCH DIGESTED	% ENZYME RECOVERED	pH OF RECOVERED FLUID
1	C	3	100	100	190	1.27	0.64	21.3		6.8
	T	3	100	100	180	1.31	0.66	22.0	95	6.9
2	C	4	100	100	188	1.36	0.68	17.0		6.8
	T	4	100	100	158	1.26	0.63	16.0	93	6.8
3	C	4	70	100	154	0.09	0.05	1.9		6.6
	T	4	70	100	138	1.04	0.52	18.5	61	7.2
4	C	3	100	100	184	0.18	0.09	3.0		7.1
	T	3	100	100	150	1.17	0.59	19.6	65	7.1
5	C	3	100	100	170	0.14	0.07	2.5		7.1
	T	3	100	100	166	1.26	0.63	21.5	84	7.0
6	C	3	100	100	185	0.15	0.07	2.5		7.0
	T	3	100	100	160	1.22	0.61	20.5	76	6.8
7	C	3	100	100	170	0.24	0.12	4.0		7.6
	T	3	100	100	150	2.28	1.14	38.0	72	7.6

*As glucose.

Results.—The results of six such experiments are summarized in Table V. It will be seen that with the exception of the first two experiments, in which pancreatic amylase must have been present in the segment because of inadequate initial washing, starch digestion affected by succus entericus amylase is very limited. It is also seen that taka-diastase is very active in the intestine. Furthermore, the taka-diastase survives one hour in the intestine with only limited loss of activity.

EFFECT OF TAKA-DIASTASE ON TOTAL DIGESTION OF STOMACH PLUS SMALL INTESTINE

Gastrointestinal digestion has been studied in one patient with a well-established ileostomy. In such a patient the ileal discharge represents the material which would normally pass into the colon and there be available for bacterial degradation. Such a patient offers an ideal "experimental preparation" for the study of factors affecting the quantity of carbohydrate reaching the colon.

Methods.—After hospitalization the patient was placed on a standard weighed diet supplying carbohydrate 177 Gm., protein 66 Gm., and fat 172 Gm. for a period of six days. During the first three-day period he received the unaugmented diet. During the second three-day period he received 2 Gm. of taka-diastase, taken in water, with each meal. The entire output of the ileostomy was collected during the entire experimental period. The material was transferred as soon as excreted to airtight bottles containing sufficient alcohol to make the final concentration 20 per cent. The wet weight, dry weight, starch and nitrogen content of the material were determined for each twenty-four-hour period. Amylase assays at pH 7.2 (pancreatic amylase) and 4.5 (taka-diastase) were carried out during the last two days of taka-diastase therapy.

TABLE VI

EFFECT OF TAKA-DIASTASE ON STARCH AND NITROGEN CONTENT OF MATERIAL DISCHARGED FROM ILEOSTOMY

DATE	WET WEIGHT FECES	DRY WEIGHT FECES	TOTAL STARCH	TOTAL NITROGEN	AMYLASE (WILLSTÄTTER UNITS 1 C.C.)	
					pH 4.5	pH 7.2
10/29	706	36	2.7	1.50	–	–
10/30	641	44	3.8	1.81	–	–
10/31	878	56	4.8	2.12	–	–
11/1*	726	56	6.0	1.92	–	–
11/2*	706	48	4.8	1.59	0.45	1.8
11/3*	746	44	2.1	1.70	0.55	1.8

*Taka-diastase (2 Gm.) with each meal.

Results.—The results of this study are summarized in Table VI. It will be seen that taka-diastase had no effect either on the nitrogen or on the starch lost in the ileal fluid. This is not remarkable when one notes that even without taka-diastase the amylolytic activity of the collected material was high, as demonstrated by the assay, at pH 7.2 where the effect of the pancreatic amylase would dominate that of the taka-diastase. In other words, the normal pancreas supplies sufficient secretion to maintain potent amylolytic power in the ileal contents. It appears, therefore, that the same fraction of ingested starch that escapes digestion by pancreatic amylase also escapes digestion by taka-diastase.

DISCUSSION

It should be clearly understood that the subjects used throughout the experiments on gastric digestion were all healthy young adults, in whom there would be no reason to suspect that enzyme supplementation would be of any practical value. These experiments were undertaken to determine whether conditions in the human stomach are compatible with effective hydrolysis by taka-diastase. It would not be expected that an enzyme preparation would markedly augment digestion in the *normal* stomach. These points are emphasized because, in a sense, this type of experimentation presents the case for enzyme therapy in its least favorable light. Enzyme therapy is a form of substitution therapy and should be reserved for cases in which an enzyme deficiency, whether on the basis of organic disease or poor hygiene, is suspected.

The fact that taka-diastase had no effect on protein digestion is, of course, not unexpected. Taka-diastase possesses only feeble proteolytic activity. The

problem was investigated for two reasons: First, because, theoretically, by liquefying the starch paste adherent to the protein particles it seemed possible that greater surface area would be exposed to the action of pepsin with consequent greater digestion. Second, it will be seen on examining Tables I and II that almost uniformly the pH was higher when taka-diastase was taken with the meal. When this phenomenon was encountered in the earlier experiments, it was interpreted as possibly being due to greater buffering power resulting from more complete hydrolysis of the protein. As a result, protein as well as starch digestion was investigated in the subsequent experiments. Since the experimental results failed to substantiate the theory, a satisfactory explanation for the pH observations is wanting at the present time. Taka-diastase has essentially no buffering power, and it seems unlikely that it actively inhibits acid secretion.

The difference between the whole meal and the finely divided meal with respect to the magnitude with which taka-diastase supplemented "normal" digestion is probably a result of two factors. First, it will be noted (Tables I and II) that with the unground meal the pH of the gastric contents fell more slowly. This permitted the ptyalin to act for a longer period so that, unaided, "maximum" digestion could be effected. Second, it appears likely that the more appetizing unground meal constituted a more potent stimulant for salivary secretion so that larger quantities of ptyalin were available for digestion.

When aided by taka-diastase, gastric starch digestion in the dog was more complete than it was in man. This is probably due to the fact that in the dog the pH of the gastric contents fell more slowly, thus permitting the taka-diastase to act for a longer period.

Since in the human studies and in the one dog (No. 2) in which acid secretion approximated that in man only negligible quantities of taka-diastase were recovered from the stomach in an active form, it must be concluded that when administered in this manner the effect of taka-diastase is largely confined to the period that it remains in the stomach. However, as has been demonstrated by Ivy, Schmidt, and Beazell,[4] when half the dose of diastase is administered on a full stomach, a large fraction reaches the intestine in an active form. The studies on the effect of taka-diastase on digestion in the intestine show that the fraction which escapes inactivation in the stomach continues to be effective.

The failure of the administered diastase to modify digestion in the patient with an ileostomy could be anticipated. Both the total nitrogen and the total carbohydrate of the material recovered from the ileostomy corresponded with normal values for feces. In other words, in this patient, by the time the terminal ileum had been reached digestion was carried to completion. Thus, supplementation of the digestive ferments could not have an effect.

SUMMARY AND CONCLUSIONS

The effect of supplementary amylase on gastric digestion was studied in "normal" human subjects and in dogs with duodenal fistulas.

1. When the tests on the human subjects were conducted with an average mixed meal, it was found that taka-diastase had no effect on digestion unless

an artificial salivary insufficiency was induced by having the subjects bolt the meal and then refrain from swallowing saliva. Under these latter circumstances taka-diastase augmented gastric starch digestion to a significant extent in 3 of 5 subjects. This meal was recovered from the stomach by voluntary regurgitation on the part of the subjects.

2. With a finely divided mixed meal that could be recovered from the stomach by means of an Ewald tube, taka-diastase effectively augmented starch digestion in all subjects with a 2 Gm. dose, and in 5 of 8 subjects with a 1 Gm. dose. With this meal it was unnecessary to induce a salivary deficiency in order to demonstrate the effect of the enzyme.

3. In the dog the average starch digestion without taka-diastase was 7.58 per cent and with taka-diastase it was 38.9 per cent. The marked difference between the control and taka-diastase experiment is due to the failure of the dog to secrete ptyalin in its saliva. In this respect, results in the dog are indicative of what might be expected in a patient with a marked ptyalin insufficiency.

4. Taka-diastase had no significant effect on gastric protein digestion in the human subjects.

5. Taka-diastase had no significant effect on the rate of emptying of the stomach.

6. In the experiments on the human subjects the quantity of taka-diastase recovered from the stomach in an active form was inconsequential. In the dog, the quantity of taka-diastase evacuated from the stomach in an active form was a function of the rate of secretion of acid by the stomach. This shows that if it is desired to have the enzyme reach the intestine in an active form, it should be administered in water at the completion of the meal, as was shown by Ivy, Schmidt, and Beazell.[4]

The effect of taka-diastase on starch digestion in the intestine was studied by introducing a solution containing a known quantity of starch with and without taka-diastase into an isolated intestinal loop of an anesthetized dog, withdrawing it at the end of an hour, and determining the quantity of reducing sugar that had been formed.

1. Starch digestion without taka-diastase was very limited (maximum 4 per cent). In the presence of taka-diastase from 16 to 38 per cent of the starch was reduced to sugar in the one-hour period.

2. The taka-diastase survived one hour in the intestine with only a limited loss of activity.

Gastrointestinal starch digestion was studied in one patient with a well-established ileostomy. The patient was hospitalized, placed on a standard diet, and the entire discharge from the ileostomy was collected for a period of six days. During the last three days of the experimental period the diet was supplemented with 2 Gm. of taka-diastase taken in water with each meal.

1. Taka-diastase had no effect on the quantity of nitrogen or starch lost in the ileal discharge.

2. The potency of the pancreatic amylase in the ileal fluid was about three times as great as that of the taka-diastase.

3. It appears, therefore, that the fraction of ingested starch that escapes digestion by amylopsin also escapes digestion by taka-diastase.

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EXPERIMENTAL ASTHMA: TREATMENT WITH HISTAMINASE*

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INTRODUCTION

ALTHOUGH most of the major principles of the induction, symptoms, and pathology of experimental asthma in guinea pigs have been investigated, the use of such a model in the evaluation of therapeutic agents has been neglected. The widespread usage of the preparation histaminase or "Torantil"† on a variety of allergic diseases led to such an experimental trial.

The advantages of experimental asthma over anaphylactic shock as a means of studying allergic phenomena are as follows: First, antianaphylaxis does not occur; i.e., animals will react over and over again in a predictable manner with no diminution in sensitivity. This has been repeatedly shown by all investigators in the field and emphasized by Manteufel and Preuner,[1] Kallós and Pagel,[2] and Ratner,[3] and is confirmed below. Thus, the use of the large numbers of animals necessary for any experiment in anaphylaxis is obviated, since an asthmatic animal may be put to repeated tests if a suitable control period is carried out before and after each test. Secondly, the reaction of an animal during an episode of experimentally induced allergic asthma bears a closer resemblance to asthma in man that does anaphylaxis. Furthermore, the degree of severity of the attack is roughly measurable. Finally it produces characteristic pathologic changes in the lungs, simulating the lesions seen in human beings.

Busson[4] in 1911 is said to have been the first to produce asthma by inhalation experiments. Following a number of successful and unsuccessful results

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by several workers, Alexander, Beeke, and Holmes[5] in 1926 caused death by inhalation of horse dander extract in guinea pigs previously sensitized either by inhalation or by intraperitoneal injection. They found that strong antigens were necessary, and even then a number of their animals failed to have any reaction. Ratner and co-workers[6] in 1927 produced sensitization, shock, bronchial asthma, and death by nasal inhalation of dry horse dander in guinea pigs. They believed that dry antigen reproduced human conditions better than liquid antigen. Manteufel and Preuner[1] in 1933 called attention to the fact that the spray droplets must be very small in order to produce asthmatic attacks in guinea pigs, and believed that previous failures were due to this fact. They stressed the absence of antianaphylaxis and produced asthmatic attacks repeatedly.

Kallós and Pagel[2] published in 1937 the results of a series of investigations on experimental asthma. These authors compared the reactions of guinea pigs subjected to allergic asthma and to inhalation of histamine and of acetylcholine. They described the clinical nature of the attacks, demonstrated the absence of antianaphylaxis, showed hereditary transmission of bronchial sensitization, and gave descriptions of the pathology showing certain differences between the three groups of animals. Finally, they demonstrated the ability of atropine, adrenaline, and a calcium preparation to inhibit the attacks.

Recently, Preuner[7] continued his studies and has reported the results of investigations made to learn the effect of the weather on experimental bronchial asthma. By grading the reaction of his animals and recording his results statistically, he concluded that changes in the weather had the effect of increasing the severity of the attack. The effects, however, of change of weather were relatively slight, but his observations established the possibility of measuring the severity of the attack. Ratner's[3] recent review is comprehensive, and he has worked out the time element in sensitization by inhalation of dry antigen.

The question as to whether release of histamine is responsible for anaphylactic shock and allergic phenomena cannot be reviewed here, but some of the more recent evidence is summarized by Laymon and Cumming.[8]

HISTAMINASE

The literature concerning histaminase has been accumulating rapidly since the description by Best and McHenry[9] of a substance in animal tissues capable of inactivating histamine when incubated with it for twenty-four hours at 37° C. The substance was observed to have the properties of an enzyme and was found in almost all organs but was most abundant in the intestinal mucosa and the kidney.

Felix[10] found that histaminase had a questionable therapeutic effect on skin diseases and stated that it failed to reduce the gastric acidity of either normal or ulcer patients. Adelsberger[11] and Ertl[12] reported favorable results in such allergic diseases as eczema and hay fever. Foshay and Hagebusch[13] found it of definite value in serum sickness. Roth and Horton[14] have used it effectively in the treatment of cold allergy.

Other reports are not so promising. Laymon and Cumming[8] found histaminase to be beneficial in only certain cases of urticaria. Kile and Rusk[1]

found no improvement in cold allergy after histaminase. A recent article denying the clinical value is that of Miller and Piness,[16] who tried it on a large number of patients with varying allergic disorders. Keeney[17] found no relief of hay fever after its use.

The experimental basis for the use of histaminase has been meager, and its toxicity has not been fully investigated. Karady and Browne[18] reported almost complete prevention of histamine shock in guinea pigs when histaminase was given intravenously fifteen minutes before the administration of a shocking dose of histamine, and a similar result was obtained in inhibiting anaphylactic shock due to egg white in a group of 30 animals. Of 10 animals as controls, 4 died; of the 20 pretreated with histaminase, none died when the homologous antigen was reinjected. The guinea pigs were given 3 units of histaminase intravenously while under ether anesthesia. However, ether anesthesia has been observed to inhibit anaphylactic shock.[19] Corper and Cohn[20] were not able to demonstrate any effect of histaminase on histamine intoxication, tuberculo-anaphylaxis, or tuberculo-allergy.

Inasmuch as the clinical picture presented in animals subjected to experimental allergic asthma is very similar to that seen in human beings with asthma, it was decided to study the influence of histaminase on the experimentally produced disease, where conditions may be more carefully controlled than in patients.

ALLERGIC ASTHMA

Following a modification of Kallós and Pagel's[2] methods, young guinea pigs, weighing approximately 10 Gm., were sensitized with 3 intraperitoneal injections at two-day intervals of 0.5 c.c. of fresh egg white diluted 1:5 with distilled water. Ten to fourteen days after the last injection the animals were sprayed with a 10 per cent solution of egg white in normal saline while in a double chamber. The chamber consisted of an inner open glass jar having a capacity of approximately 6 liters placed in a rectangular metal box measuring 77 by 46 by 46 cm. The material was sprayed directly into the inner chamber from which it passed into the larger box. The latter was provided with a small outlet tube. The vaporizer used was a painter's airbrush connected with a compressed air pump. The airbrush was adjusted to give its finest spray, which at the same time was abundant and was such as to vaporize about 5 c.c. of the fluid during a ten-minute period. The animal was sprayed directly and watched from minute to minute.

Symptoms.—Most of the animals developed asthmatic symptoms within three minutes after beginning the spray; the attack became maximal after five to ten minutes, continued as long as the spray was continued, even as long as an hour, and subsided in about thirty minutes following withdrawal from the chamber.

A typical reaction was as follows (numbers refer to the grade or degree of reaction used in recording): The first symptoms were motor unrest, ruffling of the fur, sneezes, and scratching the nose (1+). This was followed by a definite increase in the respiratory rate, more frequent sneezes, and onset of cough, usually paroxysmal and violent. The animal often chewed as if salivating (2+).

The cough would continue, although sometimes it subsided, but now in addition to the increased respirations definite dyspnea became manifest; the accessory muscles of respiration came into play, the abdominal muscles were quite active, the alae nasi would dilate, and the expiration was often prolonged. During this stage the animals defecated and urinated (3+). Finally during the most severe and usually maximal phase, respirations were labored, and now slower than in the previous stage, the whole body jerked with inspiration; deeper gasps occurred, coming at irregular intervals. Cyanosis was sometimes perceptible in the ears (4+).

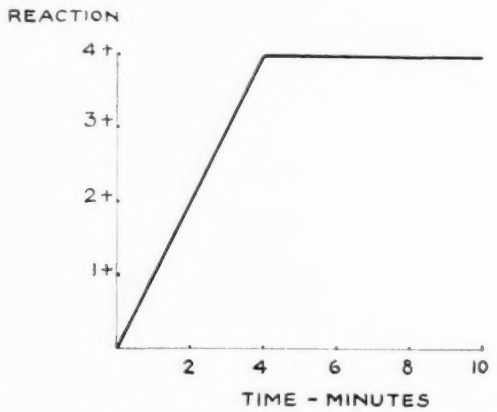

Fig. 1.—Allergic asthma: onset and course of a typical attack.

Some of the animals developed a mild conjunctivitis with lacrimation and edema of the eyelids; this usually persisted after all other symptoms had subsided. Musical whistles and squeaks were audible by stethoscope, and there was some roughening of the respiratory sounds. If the animals were graded by the afore-mentioned numbers, the usual course of events is shown in Fig. 1.

Not all the animals responded alike; in some the onset was more abrupt and in others very slow respirations developed. None of the animals died.

Differential blood counts before and after an attack showed a slight increase of eosinophiles and basophiles within twenty minutes.

Corroborating the work of previous investigators, no antianaphylactic phenomena or desensitization due to repeated attacks was found to occur, even in an animal subjected to 21 attacks of asthma at intervals of from one hour to several days.

Pathology.—One animal was sacrificed immediately after its second attack of asthma. Both attacks were maximal, and one month apart. The changes were presumed to be acute, since the animal had had no exposure or symptoms in the interval. Grossly, the lungs filled the entire chest cavity; there was some stringy material in the large bronchi, a few small subpleural hemorrhages, and on section the cut lung surface presented a few areas of reddish discoloration.

Histologically, the most striking change was a wreathing of the bronchi of all sizes with large numbers of eosinophiles, but in addition there were numerous polymorphonuclear cells, and a few lymphocytes and mononuclear cells in an

edematous peribronchial tissue. The capillaries in this area were often plugged with polymorphonuclear cells, and the endothelial cells were swollen and rounded. The bronchial epithelium was swollen and vacuolated; eosinophiles had wandered through the epithelium and were found in moderate numbers in the lumen. The latter was partially filled with mucin, desquamated epithelial cells, red blood cells, and precipitated protein. The bronchioles were often plugged with a similar exudate. Acute emphysema was manifested by stretched and ruptured alveolar walls. Occasional septal accumulations of polymorphonuclear cells, lymphocytes, and eosinophiles were noted. Small areas of atelectasis were also present, with dilated blood-filled capillaries. A few small localized alveolar hemorrhages were present. No eosinophilic pneumonia was seen. Neither true Charcot-Leyden crystals nor Curschmann's spirals were found. Sections of the liver, spleen, heart, and intestine were normal.

HISTAMINE ASTHMA

Another group of 7 guinea pigs was subjected to a total of 21 separate sprayings with 0.1 per cent of histamine phosphate at intervals of from one to five days. The symptoms were in a general way similar to those of the sensitized animals sprayed with egg white, but there were notable differences. The onset was usually much more abrupt, severe, and uncontrollable. Some of the animals reacted within three minutes; others not until fifteen minutes had elapsed. Once the reaction began, its progression was very rapid and following severe dyspnea, shock developed with apnea, deep cyanosis and spasms, and the animal fell on its side. The animal appeared moribund and the spray was discontinued. The recovery was equally as rapid as the development of the shock, occurring in three to five minutes. If the animals were sprayed again, the same severe symptoms recurred. The spray was diluted by degrees in the hope of producing a nonshocking reaction resembling allergic asthma, but the animals gave either no reaction or a very severe one. It was almost impossible to produce a definite predictable reaction from day to day as in the allergic asthma. The method thus presented no advantages over parenteral histamine shock, and was deemed unsuited for evaluation of therapeutic agents.

Kallós and Pagel[2] emphasized the differences between histamine and allergic asthma, the most notable difference being the scarcity of eosinophiles in the former. We found that the differences were one of degree rather than of kind, and they were not marked. Grossly, the lungs were again overdistended and showed a few scattered petechiae. A little frothy mucinous material was present in the large bronchi. Histologically, emphysema was again marked, with areas of atelectasis. Peribronchial eosinophilia was present but not quite so intense as in the other animals; the numerous polymorphonuclear cells seen in allergic asthma failed to occur. Hemorrhages were less frequently found. There was, however, edema of certain alveolar septa. Other organs exhibited no changes, but the peribronchial lymph nodes were heavily infiltrated by eosinophiles.

HISTAMINASE TREATMENT OF ALLERGIC ASTHMA

Histaminase or "Torantil" is a yellowish white powder supplied in ampoules of 1 histamine-detoxifying unit (1 unit of histaminase is that amount which

will inactivate 1 mg. of histamine at 37° C. in twenty-four to forty-eight hours). It is soluble in saline.

In testing the preparation, the first step was the determination of its activity in vitro. The drug was weighed out in portions equivalent to 1.0 unit and 0.7 unit. Each quantity was added to 49 c.c. of phosphate buffer at pH 7.35. One milligram of histamine phosphate in 1 c.c. of water was added to each of the two solutions, and to a control solution of buffer alone. The three solutions were saturated with oxygen, sealed and placed in a mechanical shaker in the incubator at 37° C.

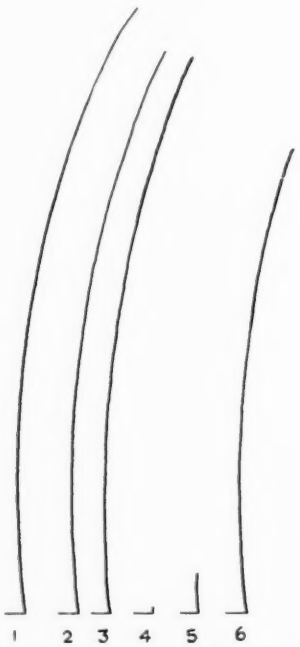

Fig. 2.—Effect of histaminase (Winthrop's Torantil) on histamine contraction of intestinal strip. *1*, 0.3 c.c. 1:10,000,000 histamine; *2*, 0.3 c.c. 1:10,000,000 histamine; *3*, 0.3 c.c. 1:10,000,000 histamine incubated 48 hours at 37° C.; *4*, 0.3 c.c. 1:10,000,000 histamine from histamine-histaminase mixture (1 mg. histamine + 1 unit histaminase) incubated 48 hours at 37° C.; *5*, same as *4*, using 1 mg. histamine + 0.7 unit histaminase; *6*, 0.3 c.c. 1:10,000,000 histamine.

The solutions were tested upon the isolated intestinal strip of the guinea pig. At the end of twenty-four hours there was almost complete inactivation in the 1 unit histaminase mixture, and in forty-eight hours it was complete (Fig. 2). It was thus seen that the drug was active in vitro according to specifications.

METHOD OF TREATMENT

Twelve guinea pigs which had been found to develop allergic asthma of a predictable degree were used. Eight of them were given intravenous control injections of saline while under ether anesthesia. Fifteen minutes later, having recovered from the effects of the anesthesia, they were sprayed in the usual fashion.

After an interval of several days, during which time they were again sprayed and found to develop asthmatic attacks equal in severity to the reactions prior to the saline injection, these 8 animals, and 4 others, were injected

intravenously with 3 units of histaminase in 2 c.c. of saline. Fifteen minutes later they were sprayed in the usual fashion, and the severity of the reactions was recorded at two-minute intervals for ten minutes. Twenty-four hours following injection of the histaminase, 6 of the group were sprayed again to detect any delayed effect.

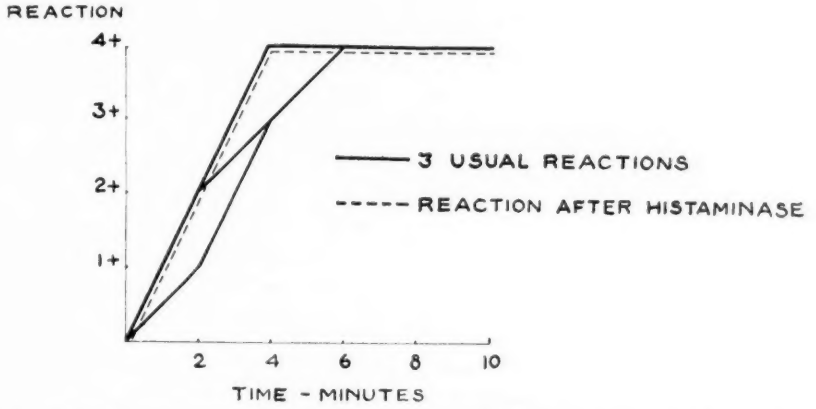

Fig. 3.—Effect of histaminase on onset and course of the typical allergic asthmatic reaction.

RESULTS

The result of a typical reaction in one animal, showing its onset and course before and after histaminase, is seen in Fig. 3. The results of all animals is seen in Table I.

TABLE I

EFFECT OF INJECTION OF HISTAMINASE ON ALLERGIC ASTHMA IN GUINEA PIGS, TOGETHER WITH CONTROLS RECEIVING ONLY SALINE

GUINEA PIG NUMBER	TOTAL NUMBER OF ATTACKS	USUAL REACTION	REACTION AFTER SALINE	REACTION AFTER HISTAMINASE	
				15 MIN.	24 HR.
33	7	++++	++++	++++	++++
34	7	++++	+++	++++	++++
35	7	++++	++++	++++	++++
36	7	++++	++++	++++	++++
37	7	+++	++	++	++
38	7	+++	++	++	++
19	9	++++	+++	+++	
22	8	++++	+++	++	
9	21	++++		+++	
17	6	++++		++++	
23	4	++++		++++	
24	4	++++		++++	

It is seen that 5 animals, Nos. 34, 37, 38, 19, and 22, had a lessening in the severity of the attack of one degree after the intravenous injection of the saline solution alone, while 4 animals, Nos. 37, 38, 19, and 9, had a similar diminution in the attack after treatment with histaminase. One animal, No. 8, had a 2+ reaction after histaminase, when its usual reaction was 4+. It would appear from the results with saline alone that either the ether anesthesia or the saline is the cause of this slight decrease, which is itself scarcely significant. There appears to be no latent or delayed effect of histaminase after twenty-four hours,

although the number of animals so tested was relatively small. The onset and course of the allergic reaction appear unchanged by injection of histaminase. In no case was asthma prevented, and in most cases it was the same with or without treatment.

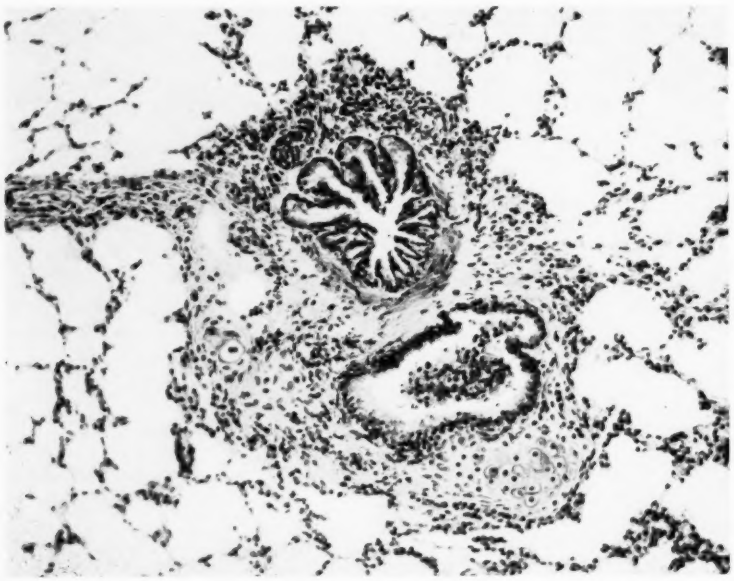

Fig. 4.—Allergic asthma.

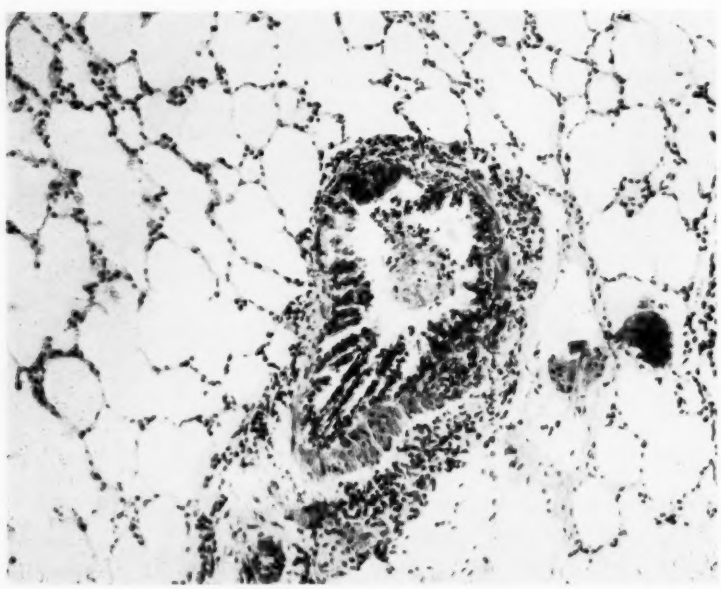

Fig. 5.—Histamine asthma.

The animals injected with histaminase did not seem to recover from the anesthesia as quickly as those injected with saline, but no severe reactions were noted on the first or second injection of the preparation, if it was in the course

of a few days. However, there were 3 animals which received histaminase about three weeks after the first injection; these animals developed severe shock which had all the characteristics of anaphylactic shock, coming on in about five minutes. One died. That this is a true sensitization to the histaminase is not surprising, since histaminase is a protein.

Thus it appears that active histaminase, when administered in amounts said to prevent anaphylaxis, fails to show activity in the prevention of experimental asthma in guinea pigs under the conditions stated.

SUMMARY

1. Guinea pigs sensitized intraperitoneally to egg white react to inhaled vaporized antigen in a predictable manner with repeated attacks and show no evidence of desensitization.

2. The symptoms of the reaction resemble human asthma, but the onset and subsidence are perhaps more rapid.

3. The pathologic picture in the lungs is characteristic and is similar in many respects to that seen in bronchial asthma in man.

4. Asthma induced by inhalation of histamine differs from experimental allergic asthma by a more abrupt onset as well as a more rapid disappearance of symptoms. It is, therefore, more difficult to control and measure. Its pathology is similar to allergic asthma but differs in degree.

5. Allergic asthma in guinea pigs is an excellent method for measuring the effect of agents on the reaction.

6. Active histaminase (3 units) given intravenously has no demonstrable effect on allergic asthma in guinea pigs.

7. Sensitization to histaminase may occur with production of anaphylactic shock upon reinjection after a suitable interval.

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ENTERAL ABSORPTION OF POLLEN ANTIGEN

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WITH the renewed interest in the use of oral pollen therapy the question of absorption has been argued pro and con. The experiments have varied with the investigator and his point of view.

Thommen[1] (1923) cited a patient with ragweed sensitivity to whom ragweed pollen was given orally. He demonstrated that the active antigen was present in the patient's blood and urine, a fact which he concludes is proof that it must have been absorbed. Black,[2] experimenting on himself, ragweed sensitive, found that the ragweed antigen was present in his blood, urine, and feces after the ingestion of the pollen extract. Each of these investigators employed sensitive patients, such as would naturally form the class of person presented to the physician for treatment, and administered daily doses to them.

Such was not the case in the experiments of several other investigators. Bernstein and Kirsner,[3] Bernstein and Feinberg,[4] London,[5] and Zeller[6] administered orally *single doses* of either ragweed pollen extract or whole ragweed pollen to *nonallergic* persons on whom local areas had previously been passively sensitized. They were unable by this method to demonstrate sufficient absorption to cause a reaction in the passively sensitized areas. Of this group of investigators, Zeller comments that by direct skin testing he was unable to demonstrate circulating pollen antigen following *either oral or hypodermic* administration of antigen. He, furthermore, admits that there were certain clinical symptoms following oral therapy which occurred with sufficient frequency that they could be accounted for only on the basis of enteral absorption.

Black, in discussing Zeller's[6] paper, states that he believes the administration of a single dose of pollen extract, even though it is large, will show little or no absorption, since most of it is excreted by the bowel. However, he points out, continued administration over a period of time does give demonstrable amounts in the blood and serum. In pursuance of this phase of the discussion it is interesting to note the report of London.[5] Contrary to the results of Thommen and Black, he was unable to demonstrate the presence of any rag-

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weed antigen in sera collected from several of his experimental patients who had taken the pollen orally. Attention is called to the fact that he collected his sera the day after the ingestion of the pollen, whereas Thommen and Black collected theirs on the day of the ingestion. Black notes particularly that after twenty-four hours the concentration of pollen antigen in the sera was greatly reduced. I believe that the important factor is proper timing between the administration of the pollen antigen (either orally or hypodermically) and the sampling of the blood or tissues.

TABLE I

POLLEN CONCENTRATION IN TISSUES OF RABBITS WHICH HAD BEEN GIVEN RAGWEED POLLEN ORALLY

RABBIT	PATIENT	KIDNEY		LIVER		MUSCLE		CONTROL EXTRACT (100 UNITS PER C.C.)
		POLLEN	NORMAL	POLLEN	NORMAL	POLLEN	NORMAL	
1	Mrs. S.	++++	+−	++	−	++	−	++++
	Mrs. E.	++++	−	++++	−	++++	−	++++
	Mr. D.	+++++	−					+++
	Miss G.	+++	−					++++
	Mrs. B.	++++	+−					++++
	Mrs. P.	+−	+−	−	−	−	−	−
2	Mrs. S.			++++	−			++++
	Miss L.			++++	−			++++
	Mrs. W.			++++	+			+++
3	Mrs. E.	++	−	−	−			++++
	Mr. D.	+	−					+++
	Mrs. W.			+	+			+++
4	Mrs. E.	++++−	−	+++	−			++++
	Mrs. W.			++	+			+++
	Miss G.	++++	−					++++
5	Mrs. B.	++	+−					++++
	Miss G.	+	−					++++
	Mrs. Sil.					+−	−	+++
	Mrs. E.					++	−	++++
	Mrs. S.					++	−	++++
6	Mr. L.	−	+−	++−	−			+++
	Mrs. W.	+−	+	+++−	+−			+++
7	Mrs. E.	++++	−	++	−			+++
	Mrs. Sp.	++	+−	++	−			+++
	Mrs. B.	+++++	+−	+++++	+			++++
9	Mrs. Sp.					++	−	++++
	Mr. Dah.					++	+	++++
	Mr. H.			++++	+−			+++
	Mrs. B.	+++	++−					++++
	Miss G.	+	+					++++
10	Mrs. Sp.					+++	++	++++
	Mr. Dah.					+++	+	++++
	Mr. H.			+++	+−			+++
	Mrs. B.	+++++	++−					++++
	Miss G.	+++	+					++++
15	Mr. M.					+++	+−	++++
16	Mrs. B.					+	+	++
	Miss H.	+++	−			+++	+	+++−

Alperstein[7] (1940), reporting a study of patients sensitive to ragweed who had been placed on oral pollen therapy, comments that the experiments showed (a) that an appreciable amount of pollen is absorbed through the gastrointestinal tract, and (b) that reagin and allergen coexist in the circulating blood at the height of treatment.

Vaughan[8] cites the experience of a physician in connection with a severe attack of asthma suffered by a ragweed-sensitive patient after eating the meat of a pheasant. It was known that the bird's craw had been full of ragweed seed. Accordingly, the physician tested the woman with extract of normal pheasant meat and then with an extract prepared from the meat of a pheasant whose craw was full of ragweed seed. To the former the patient reacted negatively, and to the latter she reacted positively. This suggested the following experiments which I herewith report.

TABLE II

POLLEN CONCENTRATION AT VARYING TIME INTERVALS IN BLOOD SERUM OF RABBITS WHICH HAD BEEN GIVEN POLLEN ORALLY OR HYPODERMICALLY

RABBIT	PATIENT	BLOOD SERUM							CONTROL EXTRACT (100 UNITS PER C.C.)
		0 HR.	15 MIN.	½ HR.	1 HR.	2 HR.	2½ HR.	3 HR.	
6 Ragweed pollen orally	Mrs. Sil.	+						+++	+++
	Mrs. E.	++−						++++	++++
	Mrs. S.	−						++	++++
	Mrs. W.	+						+++−	+++
7 Ragweed pollen orally	Mrs. E.	++					++++		+++
	Mrs. Sp.	−					?		+++
	Mrs. B.	++−					++++		++++
8 Ragweed pollen orally	Mrs. Br.	−		−*					++++
	Mrs. M.	−		++					+++
11 Ragweed pollen orally	Mrs. Br.	−		−*					++++
	Mrs. M.	−		++					+++
12 Ragweed pollen orally	Mrs. G.	+−	++−		++	+		−	+++
	Mr. F.	−	+		+	?		−	++
	Mr. G.	?	?						+−
	Mr. R.	?	+						++
13 60,000 units ragweed pollen given hypodermically	Miss G.	?	?		+−		+		+++
	Mrs. B.	−	−				+		++
	Miss Sw.	+	+++		+++++		++++		++++
14 Ragweed pollen orally	Mrs. Ber.	+		+++					++++
16 Ragweed pollen orally	Mrs. B.	+−			++				++
17 Timothy pollen orally	Mrs. K.	+−				++			++++
	Mr. B.	+				++			+++

*Twenty-four hours later the "½ hour serum" gave an inflammatory reaction 3 inches in diameter.

EXPERIMENTATION

Rabbits (either small or large) were given defatted ragweed pollen orally. Approximately two to two and one-half hours later they were killed. The kidneys, liver, and a piece of leg muscle were removed and quickly rinsed with tap water. Each was then ground and extracted with a solution consisting of 5 per cent glycerin in normal saline, 1 c.c. of extracting fluid being used for each gram of tissue. The tissues were allowed to extract in the icebox for

twenty-four to forty-eight hours, at the end of which time they were passed through a Seitz filter for sterility. Of the 19 rabbits used in the experiment, two were normal and furnished tissues for the control. To each of the other 17 1 Gm. of pollen was given orally.

Each of the extracts, together with a normal tissue extract control and a ragweed extract control, was tested on several ragweed-sensitive persons. Likewise, some of them were tested on a normal nonallergic person. All skin tests were done by intradermal method.

The results are given in Table I. A summary of the results showed that in ragweed-sensitive patients the control tissue extract gave a *negative to a two-plus wheal without pseudopodia*, while the extracts from the tissue of the rabbits which had been given the ragweed pollen orally, gave, in most cases, a *two-plus to a four-plus reaction with pseudopodia*. The control ragweed extract gave on the average a four-plus wheal with pseudopodia. These results were so definite that there can be no question that some of the pollen antigen was absorbed.

Blood was collected from some of the rabbits just before the oral administration of the pollen and at varying time periods afterward. The sera from these specimens were separated and then tested intradermally on pollen-sensitive patients. The results are shown in Table II and indicate that the active pollen antigen appears in appreciable quantities in the blood fifteen minutes after the oral administration of the pollen, but that by two and one-half hours its concentration is decreasing. For comparison one rabbit was given subcutaneously 60,000 Noon units of ragweed pollen extract while another was given orally 1 Gm. of timothy pollen. These results also are shown in Table II.

DISCUSSION

The evidence to date indicates overwhelmingly that the active pollen antigen is absorbed in appreciable amounts when given by the enteral method. This evidence consists of (1) reports in the literature of 1,409 hay fever cases which had been treated by oral pollen therapy with definite improvement in at least 944 cases; (2) demonstration that the active antigen can be found in the blood and tissue of the rabbit and in the blood and urine of man after oral administration of pollen; and (3) positive reactions following enteral ingestion of pollen. These reactions can be interpreted only on the basis of absorption. Even the opponents of the oral method speak of reactions, and it is obviously inconsistent to cite the occurrence of these while maintaining that no absorption takes place.

The only negative evidence is that enteral absorption of pollen antigen is insufficient to produce a reaction in the passively sensitized skin of nonallergic persons, whereas such a reaction *is* produced by hypodermic injection. However, if a tourniquet is place above the point of injection, thus slowing down the rate of absorption of pollen extract, the same negative result is obtained in the latter case. Hence, it is probable that the rate of absorption of ingested pollen is too slow to induce the local speed of reaction necessary in the passive transfer tests. This is further evidence of the safety of oral pollen therapy.

SUMMARY

Experimental evidence is presented which shows that after the oral ingestion of pollen by rabbits the active pollen antigen appears in the blood and tissues, kidney, liver, and muscles.

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AGE INCIDENCE OF POSITIVE TUBERCULIN REACTIONS (MANTOUX)*

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WITH A STATISTICAL ANALYSIS BY
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THE intracutaneous test with old tuberculin Koch (Mantoux test) has been used by many workers since 1909 to determine the incidence and the distribution of tuberculous infection. It has been shown that in a given community the incidence of positive cutaneous reactions to tuberculin depends on the size of the population, whether urban or rural, the incidence of *active* tuberculous disease, and the living conditions, particularly with respect to hygienic standards and the degree of overcrowding.[1]

It has also been shown that the percentage incidence of positive reactions to tuberculin and the age at which the tuberculin test becomes positive may be decidedly different for different cities, and for similar classes of people.[2] In Vienna[3] in 1909 the incidence of positive tuberculin reactions, in individuals clinically free from active tuberculosis, was found to be very high among the poorer classes; one-half the children showed a positive reaction at 5 years of age; about 90 per cent at 10 years, and 94 per cent at puberty. In New York City the incidence was found to be considerably lower during the years 1921 to 1928 by Smith,[4] and in 1934 in a survey made by one of us (F. P.).[5] Table I illustrates the striking difference in age incidence of positive tuberculin re-

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actions in comparable sections of the populations of Vienna and New York City. The somewhat higher incidence in Smith's series as compared with the group studied by one of us (F. P.) may be explained by the fact that Smith's group included cases of active tuberculosis.

TABLE I

COMPARISON OF AGE INCIDENCE OF POSITIVE TUBERCULIN REACTIONS

AGE	VIENNA 1909 (%)	NEW YORK CITY 1921-1928 (%)	NEW YORK CITY 1934 (%)
5	50	14.7	9.5
10	90	37	29.1
12-13	94	45.5	35.2

The incidence of positive tuberculin reactions may vary with changing conditions. In the same community the lapse of a decade or a few years may influence the statistics considerably.[6] Chadwick and Zacks[7] reported a reduction of 23 per cent in positive reactions among children of the same age groups between the years 1917 and 1926, coincidental with an intensive antituberculosis campaign. It is important, therefore, when interpreting results to take into consideration the year or the period during which a survey is made.

EXPERIMENTAL

We should like to report the results of our studies in the age incidence of positive tuberculin reactions carried out in a group of 362 persons, ranging in age from a few months to 80 years.

The persons in whom the tests were made were patients treated for various dermatoses at the Skin and Cancer Unit during the year 1939. The clientele of this clinic consists predominantly of white urbanites from the more crowded districts of New York City, whose economic status may be rated as poor or lower middle class. Patients suffering from any form of skin tuberculosis were excluded from the study.

Old tuberculin Koch, obtained from the Department of Health of the City of New York, was used, and the Mantoux technique was employed. The initial test dose was 0.02 mg. of O. T. (0.1 c.c. of 1:5,000 dilution). The patients were retested with 0.1 mg. of O. T. (0.1 c.c. of 1:1,000 dilution) and 1.0 mg. O. T. (0.1 c.c. of 1:100 dilution) if the first injections failed to elicit a reaction.

The test site was examined forty-eight hours later, and the reaction was graded as one plus to four plus, as recommended by the National Tuberculosis Association.[8] The patients were then classed as belonging to four different groups, depending on the intensity of the reaction and the concentration required to elicit a reaction:

I. Strongly positive—if a three-plus to four-plus reaction was elicited with 0.02 mg. O. T. Koch.

II. Moderately strong positive—if a one-plus to two-plus reaction was elicited with 0.02 mg. O. T.

III. Weakly positive—if either 0.1 mg. or 1.0 mg. O. T. was required to elicit a reaction.

IV. Negative—if the patient failed to react to 1.0 mg. O. T.

RESULTS

In the age groups of the first two decades there was a gradual rise in the incidence of positive reactions to 33.3 per cent. Between the twentieth and twenty-ninth years the rise in the incidence of positive reactions was sharp, from 33.3 per cent to 90.9 per cent. After the thirtieth year the positive incidence remained fairly constant, varying between 90 per cent and 98 per cent.

TABLE II

FREQUENCIES OF POSITIVE TUBERCULIN REACTIONS AT VARIOUS AGES IN A RANDOM SERIES OF 362 PERSONS

AGES	NUMBER OF PERSONS TESTED	NUMBER OF NEGATIVE REACTIONS	POSITIVE REACTIONS	
			NUMBER	PER CENT*
0-4	26	26	0	0
5-9	20	19	1	5.0 ± 3.2
10-14	25	18	7	28.0 ± 6.0
15-19	33	22	11	33.3 ± 6.0
20-24	40	17	23	57.5 ± 5.1
25-29	44	4	40	90.9 ± 2.9
30-39	73	4	69	94.5 ± 1.8
40-49	50	1	49	98.0 ± 1.3
50-59	32	1	31	96.9 ± 2.0
60-80	19	2	17	89.5 ± 5.7
Totals	362	114	248	

*The figure following the ± sign represents the probable error of the observed frequency.

In Table II are listed the frequencies of positive tuberculin reactions among 362 persons at various ages, and in Fig. 1 these results are presented graphically. The observed differences in incidence of positive tuberculin reactions at varying ages are statistically significant. The probable error of an observed frequency is calculated from the formula:

$$\text{Probable Error} = 0.6745 \sqrt{\frac{p\ (1-p)}{N}}$$

where p is the observed frequency and N is the number of persons in the sample tested. This formula was applied to the frequencies obtained for positive tuberculin reactions in various age groups, the results being listed in the last column of Table I. As an example, the calculation for age group 25 to 29 is given:

$$\text{P.E.} = 0.6745 \sqrt{\frac{(0.909)\ (0.091)}{44}} = 0.029, \text{ or } 2.9 \text{ per cent.}$$

The formula is said to be accurate only for samples containing 30 or more persons, but for the sake of uniformity it was also used for age groups 0 to 4, 5 to 9, 10 to 14, and 60 to 80, each of which contained less than 30 persons. However, the error in doing this is not great, since the smallest group contained 19 persons.

The frequencies of positive tuberculin reactions in two different age groups can be compared by determining the differences between the two frequencies and then comparing this difference with its probable error. The probable error of a difference between two independent frequencies p_1 and p_2 is calculated by using the formula:

$$\text{P.E.}_{\text{Diff.}} = \sqrt{(\text{P.E.}_{p_1})^2 + (\text{P.E.}_{p_2})^2}$$

The odds that a particular difference is significant, that is, not purely accidental, is determined with the aid of Table III. The usual convention is to consider as significant any difference which is more than three times its probable error.

TABLE III

SIZE OF DIFFERENCE IN RELATION TO ITS PROBABLE ERROR	ODDS THAT DIFFERENCE IS SIGNIFICANT
± P. E.	1 to 1
± 2 P. E.	3.6 to 1
± 3 P. E.	21 to 1
± 4 P. E.	140 to 1
± 5 P. E.	1,300 to 1
± 6 P. E.	19,200 to 1
± 7 P. E.	427,000 to 1
± 8 P. E.	14,700,000 to 1
± 9 P. E.	730,000,000 to 1
± 10 P. E.	65,000,000,000 to 1

As an example, let us compare the incidence of positive tuberculin reactions in age groups 10 to 14 and 20 to 24. Here $p_1 = 28.0$ per cent and P.E.$p_1 = 6.0$ per cent; $p_2 = 57.5$ per cent and P.E.$p_2 = 5.1$ per cent.

Therefore, $p_2 - p_1 = 29.5$ per cent and P.E. $(p_2 - p_1) = \sqrt{(0.06)^2 - (0.051)^2} = 0.081$, or 8.1 per cent.

Since $p_2 - p_1$ is more than three times its probable error, this difference is probably significant. How increasing the size of the samples helps to demonstrate differences among the age groups is illustrated by pooling together the results for ages 0 to 24 and comparing these with the observations made on persons more than 25 years old. Among 144 persons under 25 years old the incidence of positive tuberculin reactions was 29.2 ± 2.9 per cent; among 218 persons older than 25 years it was 94.0 ± 1.1 per cent. Accordingly, the difference between these two groups was 64.8 ± 3.1 per cent, so that this difference was more than twenty times its probable error!

DISCUSSION AND SUMMARY

We are of the opinion that the sharp rise in the incidence of positive tuberculin reactions during the third decade, from 33.3 per cent to 90.9 per cent, may be of clinical and epidemiologic significance.

It has generally been held that in urban communities the majority of persons are infected with tuberculosis by the time adolescence is reached. Our findings, and the recent studies of others, indicate that this may not be so. Smith[4] reported a positive incidence of 45 per cent in persons from 10 to 15 years old; Drolet,[9] 29 per cent; Dickey and Seitz,[10] 37 per cent; Aronson and Nicholas,[11] 31.8 per cent. Soper and Amberson[12] state, "in this country at large, roughly speaking, 50 per cent of the white population under the age of twenty does not react to tuberculin."

If the tuberculin reaction is used as the criterion of past or present tuberculous infection, our results indicate that under present conditions in New York City the majority of persons are not infected until adult life. Long[13] has shown, however, that the tuberculin test may fail to detect all healed pri-

mary tuberculosis because of the waning of cutaneous sensitivity that occurs with healing. One cannot assume, therefore, that the rise of positive tuberculin reactions during the third decade is due entirely to initial infection with tubercle bacilli. Superinfection or reinfection of sufficient magnitude to heighten tuberculin sensitivity, spontaneous fluctuation in sensitivity, or increase of sensitivity due to maturation or other factors, must also be considered as the possible modus operandi in some cases.*

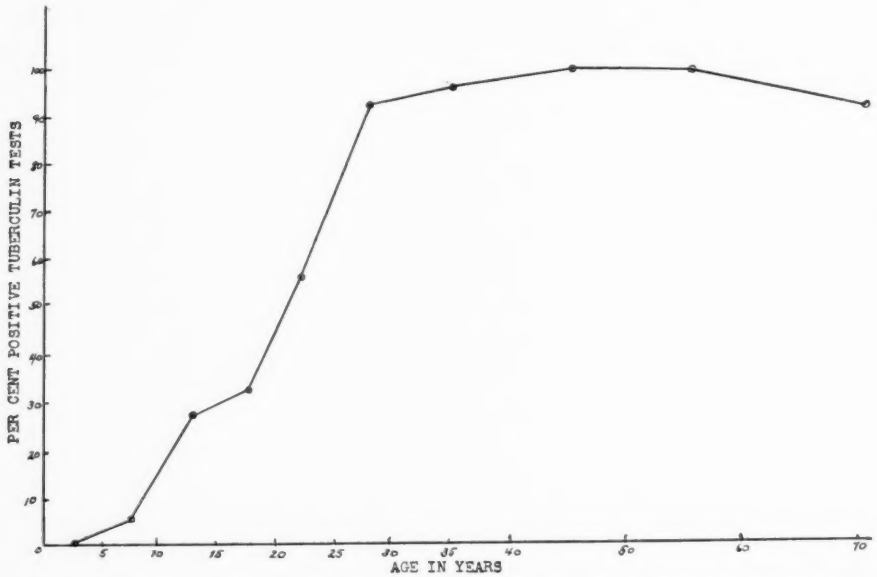

Fig. 1.—Incidence of positive tuberculin reactions at various ages based on a study of a random series of 362 persons.

The report of the New York State Department of Health (Division of Tuberculosis) in the annual report of 1937[14] confirms the clinical significance of our findings. The largest number of new cases of active tuberculous disease of all types was reported during the third decade. This finding is in striking conformity with the present demonstration of sharp increase in incidence of cutaneous tuberculin sensitivity in precisely this age group.

The limitations of the tuberculin test as a sole means of case finding have been pointed out by Plunkett,[15] and we are aware that the tuberculin test is of questionable value as a means of establishing an epidemiologic index of tuberculous infections.[16] Study of age incidence of positive tuberculin reactions may serve, however, to indicate the age groups in which an intensive search for new cases should be made.

Under the present conditions in New York City the results of quantitative tuberculin skin tests coincide with the clinical statistics, showing that for the appearance of active tuberculous disease, the third decade appears to be the most vulnerable age.

*There are many possible explanations for the 9 per cent drop in positive reactions in the seventh and eighth decades, but these cannot be entered into here.

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THE QUESTION OF PROTECTION AGAINST HISTAMINE AND ANAPHYLACTIC SHOCK IN GUINEA PIGS BY HISTAMINASE*

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THE in vitro inactivation of histamine by histaminase preparations is a slow process even under optimal conditions, as shown by Best and McHenry.[1, 2] However, Karady and Browne[3] found that histaminase preparations injected into etherized guinea pigs exerted a protective effect against lethal doses of histamine, and also against anaphylactic shock. They concluded that histaminase acts more rapidly in vivo than in vitro, and that their observations afforded experimental support for the reported therapeutic effectiveness of histaminase preparations against allergic responses in man.

Haag and Latz[4] also performed experiments which they interpreted as indicating a protective effect of histaminase against anaphylactic shock. This interpretation is not well supported by their experimental results, since, when normal guinea pigs were used, 12 showed greater symptoms when shocked twelve hours after "protection" with a histaminase preparation than did the 14 similarly sensitized and shocked controls which were not given a protective injection. Haag and Latz ignored these animals and drew their conclusions

*From the Laboratories of Dr. George Piness and Dr. Hyman Miller, Los Angeles.
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from the results of a similar test done with 29 animals which had survived a (negative) trial of the desensitizing action of multiple small doses of histaminase. Alternative explanations of the mechanism of the protective action suggested by this experiment would appear to be possible, in view of the facts that histaminase preparations contain antigenic hog proteins and that the "protective" injections were immediately followed by slight but definite symptoms suggestive of anaphylactic shock.

Our own trials of histaminase preparations in man for the treatment of allergic conditions[5] and for the control of histamine or allergic skin reactions[6] did not give any definite indication of in vivo activity or of therapeutic effect. It was, therefore, thought desirable to repeat the experiments reported by Karady and Browne.

With one exception, the observations of Karady and Browne did not include any control animals handled in the same way as those treated with histaminase (etherization and intrajugular injection). This exception consisted of three guinea pigs given heat-inactivated histaminase followed by histamine, and it was found that these died more quickly than the controls given histamine alone. Not only were these animals too few in number to serve as convincing procedure controls, but there is a possibility that heating may have produced toxic properties in the histaminase solution.

In order to simplify our technique, and to conserve our limited supply of histaminase, we compared the protective effect of histaminase solutions with control saline solutions, both solutions being injected intracardially into unanesthetized animals.

EXPERIMENTAL

A. Solutions and Procedures.

Saline. An 0.85 per cent sodium chloride solution containing 1:1,000,000 of phenylmercuric acetate as preservative.

Buffered Saline. A solution of sodium chloride, disodium phosphate, and monopotassium phosphate, having a pH 7.4, a ratio of sodium to potassium ion concentrations of 40:1, and an ionic strength equal to that of 0.85 per cent sodium chloride. Same preservative.

*Histaminase.** "Parenteral histaminase," a soluble powder, received in sealed glass ampoules. For Experiment I the contents of 42 one-unit ampules were dissolved in 28 ml. of buffered saline about two hours before use. For Experiment II, Part 1, five one-unit ampoules and 20 two-unit ampoules were dissolved in 30 ml. of saline about two hours before use. For Experiment II, Part 2, 20 ten-unit ampoules of a special experimental lot were dissolved in 27 ml. of buffered saline three hours before use. After two guinea pigs had been killed immediately (with pulmonary and pleural hemorrhages) by intracardial injection of the last solution at the usual dose of 8 ml. per kilogram, it was diluted 1:5 with buffered saline in order to bring it to the same strength (in labeled units) as the other histaminase solutions.

*Histaminase was supplied through the courtesy of the Winthrop Chemical Co. and Dr. J. B. Rice, its Director of Medical Research. The one-unit ampoules were designated "T-360-1," and the two-unit ampoules, "T-360." Samples of these were returned to the manufacturers for re-assay just before Experiment II, Part 1, was done. They reported that the "T-360-1" had dropped in potency to about 60 per cent of the labeled value, while the "T-360" retained its original potency.

Histamine. A solution containing 10 mg. of histamine dihydrochloride (Hoffman-LaRoche) per milliliter in buffered saline was freshly prepared for each experiment in which it was used.

Egg Albumen. The stock from which solutions were made is a four times crystallized preparation,[7] consisting of the washed crystal mush, pressed between blotters to remove most of the wash liquor, dried rapidly in a current of warm air, and stored in the cold. Nitrogen analyses showed that about 1.5 per cent of the egg albumen was denatured by drying, and that the total content of egg albumen was 64.2 per cent, while the content of ammonium sulfate was 31.7 per cent. For sensitization 46.7 mg. of the dry material was dissolved in 30 ml. of buffered saline (1 mg. of egg albumen per milliliter), and for shocking, 4.67 Gm. were dissolved in 30 ml. of buffered saline (100 mg. of egg albumen per milliliter).

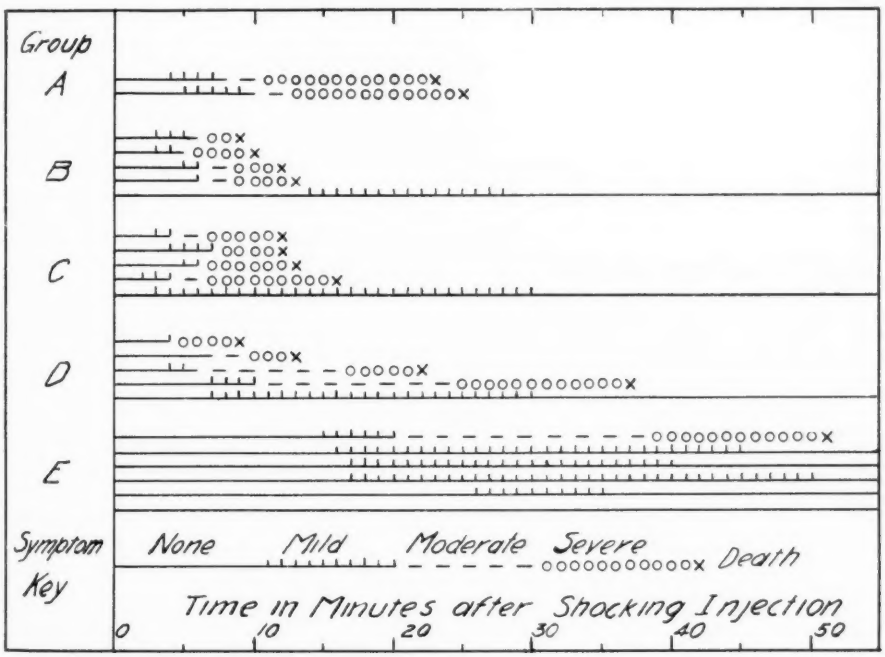

Fig. 1.—Experiment I—Histamine shock.

Group	"Protecting" Injection (Intracardial)	Shocking Injection (Intraperitoneal)
A	None	15 mg./kg. histamine dihydrochloride
B	None	25 mg./kg. histamine dihydrochloride
C	2 ml. histaminase*	25 mg./kg. histamine dihydrochloride
D	8 ml./kg. histaminase*	25 mg./kg. histamine dihydrochloride
E	8 ml./kg. buffered saline	25 mg./kg. histamine dihydrochloride

The first guinea pig in group D was depressed and uncertain on his feet between four and six minutes after the first injection.

*This histaminase solution contained, according to re-assay, approximately 0.9 units per milliliter, instead of the intended 1.5 units per milliliter.

Doses and Injection Technique. Because our guinea pigs differed in weight from those used by Karady and Browne, it seemed desirable to give the histaminase and histamine in proportion to weight. Doses of 8 ml. per kilogram of histaminase solution, and 2.5 ml. per kilogram (25 mg. per kilogram) of histamine dihydrochloride solution were selected as a fair approximation to the average dose used by them. In the first experiment, however, two guinea pigs

were given 15 mg. of histamine dihydrochloride per kilogram (group A), and one group of animals (group C) was given 2 ml. of histaminase solution without regard to weight.

Histamine solutions were injected intraperitoneally. Histaminase and control saline solutions were given intracardially, using a 1¼ inch, No. 22 gauge needle, and entering the chest through the diaphragm, inserting the needle along the left side of the xiphoid process. The solutions were injected slowly, testing for needle placement by drawing a drop or two of blood up into the syringe at the beginning and end of the injection. Judging by the color of the blood, almost all the injections were made into the left side of the heart. The animals withstood the injections very well, with the exception of the first guinea pig of Experiment I, group D. The histaminase or control saline solution was always administered fifteen minutes before the shocking injection.

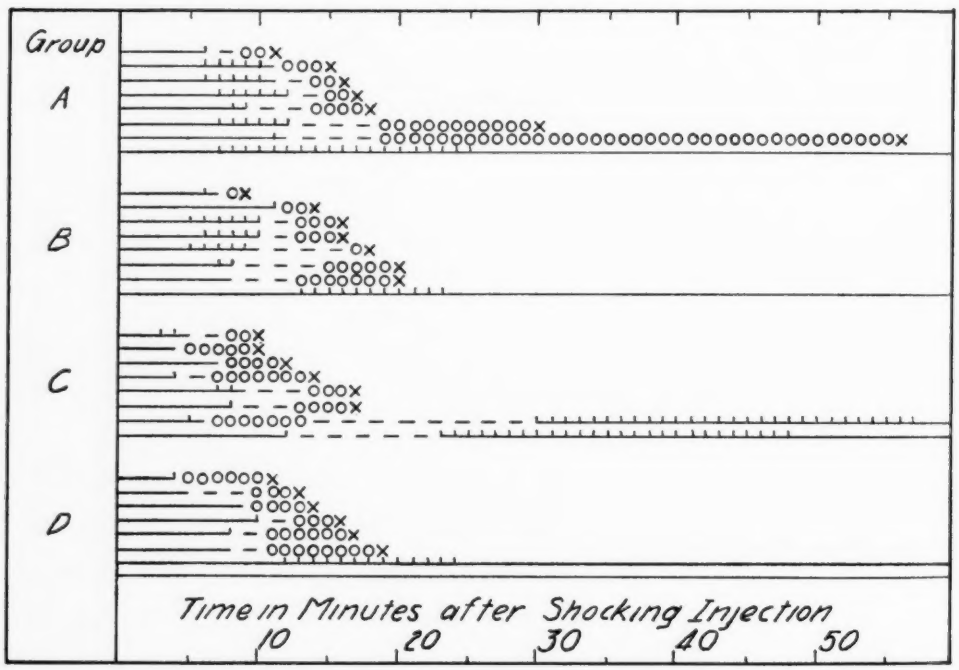

Fig. 2.—Experiment II, Part 1—Histamine shock.

Group	"Protecting" Injection (Intracardial)	Shocking Injection (Intraperitoneal)
A	None	25 mg./kg. histamine dihydrochloride
B	8 ml./kg. saline	25 mg./kg. histamine dihydrochloride
C	8 ml./kg. histaminase*	25 mg./kg. histamine dihydrochloride
D	8 ml./kg. buffered saline	25 mg./kg. histamine dihydrochloride

(For symptom key, see Fig. 1.)

*This histaminase solution contained, according to re-assay, approximately 0.9 unit per milliliter, instead of the intended 1.5 units per milliliter.

The sensitizing and shocking doses of egg albumen were given without regard to animal weight, 1 ml. of the respective solution being administered intraperitoneally. The sensitizing dose was given two days after the animals were received, and the shocking dose was administered six weeks later. To assure ourselves that the ammonium sulfate content (49.4 mg. per milliliter) of the shocking solution was not high enough to be toxic, and that the solution was otherwise free of primary toxicity, three nonsensitized guinea pigs (group

E, consisting of three of the six survivors of Experiment II, Part 1, which was done fourteen days previously) were given the shocking dose of egg albumen solution. They showed no signs of difficulty or discomfort at any time.

Animals. All guinea pigs were obtained from a single source and were uniform in age. Those for Experiment I weighed from 480 to 650 Gm. Those for Experiment II, both parts, all obtained at one time, were all white, weighed from 200 to 250 Gm. when received, from 340 to 480 Gm. when Part 1 (histamine shock) was done four weeks later, and from 400 to 510 Gm. when Part 2 (anaphylactic shock) was completed. The animals were not selected for sex. A record of sex was kept for Experiment II, but no differences were seen. The guinea pigs were fed plenty of greens, supplemented by rabbit pellets, and water was available to them at all times.

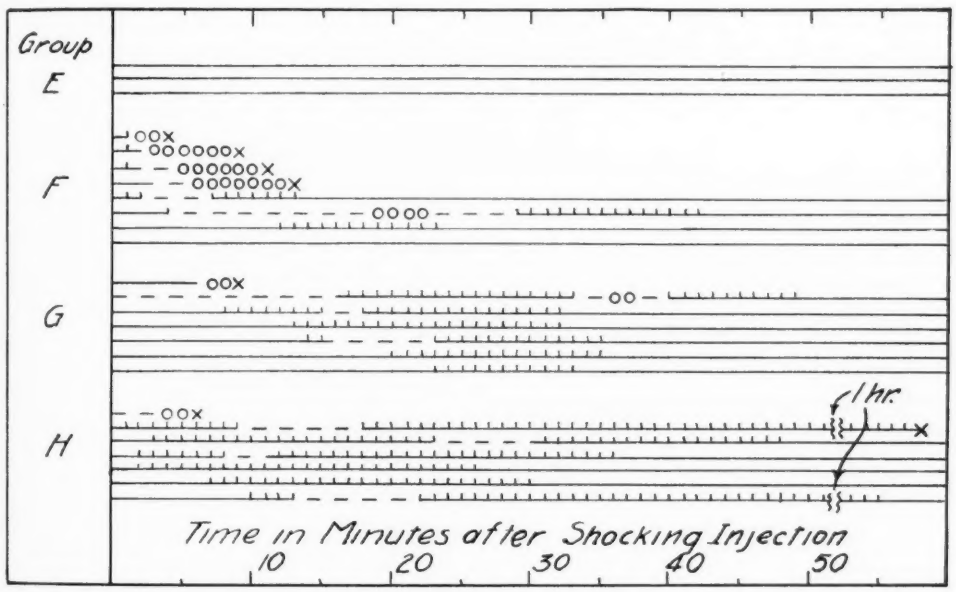

Fig. 3.—Experiment II, Part 2.—Anaphylactic shock.

Group	"Protecting" Injection (Intracardial)	Shocking Injection (Intraperitoneal)
E	(Not sensitized, see text)	100 mg. egg albumen
F	None	100 mg. egg albumen
G	8 ml./kg. histaminase*	100 mg. egg albumen
H	8 ml./kg. buffered saline	100 mg. egg albumen

(For symptom key, see Fig. 1.)

*This histaminase solution contained 1.5 units per milliliter.

B. Experiments.

The results of the experiments are shown in the diagrams: Fig. 1 for Experiment I, Fig. 2 for Part 1 of Experiment II, and Fig. 3 for Part 2 of Experiment II. Each line represents a guinea pig, and the key to the symbols is shown in Fig. 1. The symptoms were classified as mild, moderate, and severe, in the order of their usual development, as follows:

Histamine shock symptoms:

Mild: depression, lowered respiratory rate, scratching.
Moderate: respiratory difficulty, coughing, gasping.
Severe: collapse, convulsions.

Anaphylactic shock symptoms:

Mild: restlessness, ruffled fur, sneezing, scratching, loss of sphincter control.

Moderate: irregular respiration, jumping.

Severe: rearing, gasping, collapse, convulsions.

(Depression is the principal symptom after dyspnea has disappeared, when animals show signs of recovering.)

All the animals which have been used by us in this type of experiment are included in the results.

DISCUSSION AND CONCLUSIONS

A slight prolongation of survival time after histamine by histaminase solutions occurred in both C and D of Experiment I, and a marked prolongation in anaphylactic shock in Experiment II, Part 2. To this extent our data agree with the findings of Karady and Browne.[3] However, in no experiment was there any definite indication that the histaminase solution had greater efficacy than an equivalent volume of the saline solution used to dissolve the histaminase; and in Experiment I more of the saline solution group of animals survived than of the histaminase treated groups. However, none of the differences between any two groups of animals is statistically significant. Therefore, the only conclusion which may safely be drawn from the experiments is that Karady and Browne's statement that histaminase protects animals against histamine and anaphylactic shock is not supported either by the evidence they offered or by our findings. It should be pointed out that this cannot be taken as proof that histaminase preparations altogether lack in vivo activity of this nature, but only that the question remains undecided at present.

Because of the failure of any solution used in Experiment II, Part 1, to show protective action against histamine shock, we are unable to draw any definite conclusion regarding protective effect by saline injections. However, it is interesting to note that the dose of 8 ml. per kilogram is of the same relative order of magnitude as that used in intravenous saline therapy for surgical and other shock in man.

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LIPOMELANOTIC RETICULAR HYPERPLASIA OF LYMPH NODES*

Report of a Case

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BIOPSY, whenever possible, has become a routine procedure to establish a diagnosis which cannot be made with certainty by any other means. This is especially true in the difficult group of diseases characterized by lymph node enlargement. It is important to bear in mind that local lymph node enlargement may not necessarily have any relation to a systemic illness of which the patient complains. The purpose of this paper is to report a characteristic lymph node pattern which was found accidentally in a young adult who died of acute pulmonary miliary tuberculosis. This benign lesion has some superficial histologic resemblances to the malignant lymphoblastomatous and lymphogranulomatous lesions with which, indeed, it was confused until the true nature of the lesion was described by Pautrier and Woringer.[1] It is, therefore, worth while to call attention to this generally unfamiliar, although probably not uncommon, lesion.

REPORT OF CASE

K. T., a young negro boy, 17 years old, entered the Temple University Hospital on the service of Dr. Charles L. Brown on Sept. 14, 1940. He was well until June 1, 1940, when he noticed sharp stabbing pains around the heart, which lasted ten to fifteen minutes, and which were at times so severe as to cause him to faint. He was put to bed by his doctor for three weeks. At the expiration of this time he found that he was too weak to stand and was very short of breath. Three weeks before admission he developed pains in his extremities, especially the lower ones, and in his joints. Since the onset of his illness he lost 50 pounds. All during his illness he believes that he had fever, which occasionally was as high at 104° F.

Physical examination revealed a young adult negro male who had evidently lost considerable weight. There was nothing of note in the entire examination, except for the chest and inguinal lymph nodes. Expansion was limited and lagged on the left side of the chest. There was impaired percussion on the left side posteriorly from T_8 downward; this extended anteriorly to the midaxillary line. Breath sounds were absent in this region. There was moderate enlargement of the inguinal lymph nodes on both sides. These lymph nodes were discreet, not tender, and varied in size up to 5 cm. in diameter.

The laboratory findings were as follows: hemoglobin varied from 11.5 Gm. to 13 Gm. (Haden-Hausser significant). R.B.C. 4.01 to 4.83 million, W.B.C. 6,300 to 5,700. The average differential was neutrophiles 74 (N. F. 47), lymphocytes 17, monocytes 6, eosinophile 1, neutrophylic myelocytes 3. Urine was slightly positive for occult blood, 6 to 7 R. B. C. per high-power field, W.B.C. 3 to 4 per high-power field occasional coarse granular casts. The electrocardiogram revealed a simple tachycardia with low T-waves, suggestive of some impairment to myocardial function, probably secondary to the patient's general infection. During his stay in the hospital he ran a continuous fever, ranging from 99° to 105° F. X-ray examination of the chest revealed an abnormal diffuse punctate type of density throughout both lung fields, and a small fluid collection in the left pleural space. Intravenous pyelogram presented a normal x-ray appearance.

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The presumptive diagnosis was tuberculosis. However, sputum, gastric washings, urine, and cerebral spinal fluid studies were repeatedly negative for tubercle bacilli. Because of the inability to establish a definite diagnosis, it was decided to remove one of the inguinal lymph nodes for histologic study.

The lymph node removed was pigmented and slate gray in color. It was completely encapsulated. In one section at one pole there was a single island of giant cells, suggestive of tuberculous origin. The remainder of the gland had none of the characteristics of tuberculosis. There was considerable thickening of the capsule. The follicles were small and poorly defined and had been replaced apparently by a tremendous hyperplasia of the reticulo-endothelial cells. These cells were polymorphous in appearance with occasional prominent and multiple nucleoli. The most striking feature, however, was the tremendous infiltration of fat and melanin within the reticulo-endothelial cells.

The patient died on Oct. 14, 1940, and autopsy disclosed an acute pulmonary miliary tuberculosis with terminal miliary spread to the other viscera.

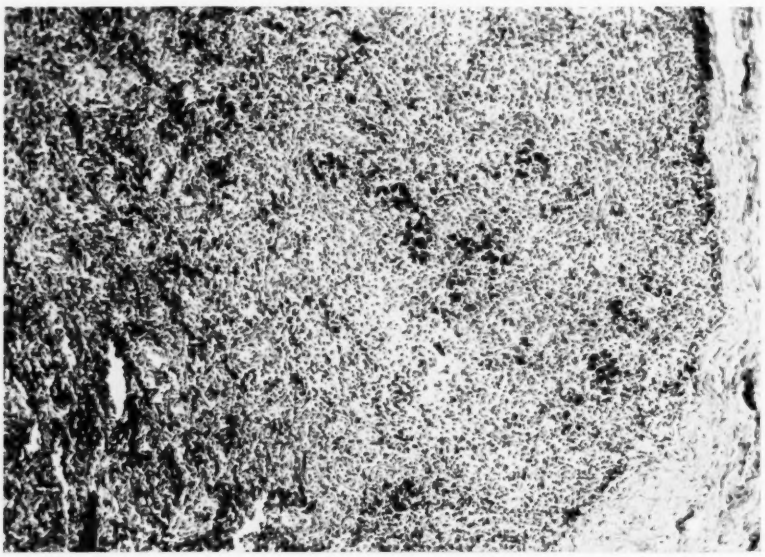

Fig. 1.—Illustrating thickened capsule, disorganization of normal architecture, marked reticulum cell hyperplasia with deposition of fat and melanin.

DISCUSSION

The major portion of the lymph node picture was not obviously due to tuberculosis. The tremendous amount of fat, melanin, and reticular hyperplasia represented a puzzling picture until the contribution of Pautrier and Woringer was discovered. They found a rather characteristic histologic picture of lymph nodes in a variety of dermatologic diseases, the common denominator of which was pruritus. They called this lesion "La reticulose lipomelanique." The description of this lesion is taken rather freely from their contribution.

The lymph nodes are normally encapsulated and free of adhesions. They are moderately enlarged up to the size of a pigeon's egg. Upon sectioning, the cortical zones appear clearer than normal, with scattered brown flecks representing melanin deposits. Histologically, the lymph node tends to retain its fundamental architecture. The lymph follicles are smaller than normal and

are separated farther apart than normal. This is due to the interfollicular reticulum cell hyperplasia, which causes a gradual reduction and ultimate disappearance of the secondary lymph follicles. The germinal follicle, however, remains active and may be larger or smaller than normal, depending upon the extent of reticular hyperplasia. The reticular cells are predominantly cortical in position, although with increase in production, they may invade the medulla. The reticular cells are large pale cells with indefinite cytoplasmic borders. The cytoplasm appears faintly vacuolated with an empty meshwork. The nuclei are large, clear, elongated, or irregularly oval, with sprayed chromatin and occasionally two prominent nucleoli. These cells contain large quantities of lipoid substances easily stained with sudan III or scharlach R and are, according to

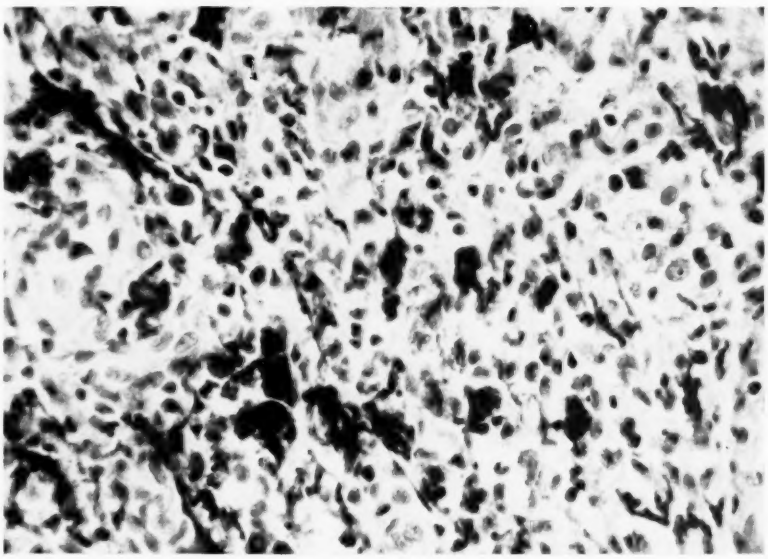

Fig. 2.—High power. Illustrating the polymorphous reticulum cell hyperplasia. The black deposits represent melanin. The clear spaces represent fat.

Pautrier and Woringer, cholesterol and double refractile. In addition, in the peripheral reticular cells especially are deposited large amounts of brown pigment, which by staining can be identified as melanin. The entire vascularity of the gland is increased. The marginal subcapsular sinuses are dilated and free for lymphocytic circulation. Eosinophiles may be found in them.

Pautrier and Woringer interpreted the hyperplastic proliferation of the reticular cells as due to an attempt of these cells to engulf and dispose of the excessive lipoid substances and melanin. They found these lesions in persons subject to a variety of skin diseases associated with pruritus. Normally, melanin, which is elaborated by the melanoblasts in the basal layer of the skin is expelled toward the surface by the normal process of keratinization. A minute histologically undetectable quantity may travel toward the regional lymphatics and ultimately be excreted by the kidneys, and perhaps by the intestines. With scratching induced by itching, more than a normal quantity of melanin is formed. The regional lymphatics are dilated by the irritation. These factors, in addition to the massaging action of scratching, help to drive excessive quan-

tities into the regional lymphatics, where it is trapped by the reticular cells and causes them to multiply. They believe that the lipoid process is similar. The amount of melanin deposition varies with the duration and the severity of the skin lesion. It probably also varies with the melanin content of the skin.

In the absence of considerable melanin deposition, the lymph node may bear a striking resemblance to Hodgkin's disease. Dorothy Reed cells are not present, but the reticular cells are highly polymorphous and voluminous at times. The nucleoli are prominent and may be multiple; eosinophiles, plasma cells, and neutrophiles may be plentiful in the subcapsular lymphatics; fibrosis may be present in the older lesions, and a partial or complete disorganization of the lymph node architecture appears. Indeed, the diagnosis of Hodgkin's disease, mycosis fungoides, and reticulum cell sarcoma had been made on many tissues which Pautrier and Woringer later diagnosed as "La reticulose lipomelanique."

After histologic examination of the lymph node, the patient was asked specifically if he ever had any skin disorders. He stated that for several years he has had itching, occasionally scaling lesions in both groins for which numerous remedies had been tried unsuccessfully. These lesions were thought to be due to his occupation, which was that of pressing suits with a steam pressing machine. This required him to place one foot alternately on a foot stool. Apparently the heat and friction were sufficient to set up this irritation. He frequently attempted to obtain relief from the itching by scratching. He had been doing this work irregularly for the preceding four years and about two years before, he noticed the appearance of "lumps" in his groins.

CONCLUSION

A specific lymph node picture distinguished histologically by a marked reticulum cell hyperplasia rich in fat and melanin occurring in a variety of diseases characterized by itching is reported.

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LABORATORY METHODS

GENERAL

THE OPSONOCYTOPHAGIC TEST IN ACUTE DIARRHEA IN INFANTS AND CHILDREN*

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FOR several years extensive bacteriologic studies have been made of the stools of infants and young children who were admitted to the Children's Hospital of Cincinnati suffering from acute diarrhea. Stools from 50 per cent of these patients have contained *Shigella paradysenteriae*[1] (dysentery bacilli), but repeated stool examinations have offered no information regarding the etiologic factor in the patients from whose stools *Shigella paradysenteriae* could not be isolated. It was hoped that a determination of the opsonocytophagic power of the blood of such patients for the organisms isolated from their stools might indicate a possible causal relationship between the presence of these organisms and the disease. This theory was not substantiated by observation in our group of patients—high indices being obtained from patients both with and without diarrhea. The results from the patients with dysentery, however, were of considerable interest and, accordingly, are being reported here.

METHODS

The method employed for studying the opsonocytophagic index was for the most part an adaptation of the method of Huddleson, Johnson, and Hamann,[2] and consisted of mixing equal quantities of citrated whole blood and standardized formalin-killed bacterial suspension in agglutination tubes, incubating for thirty minutes at 37° C., mixing, streaking as for a differential blood count, staining with dilute crystal violet, and determining the number of organisms in fifty polymorphonuclear leucocytes. Whole blood was used throughout these studies, because previous investigations by others[2, 3] indicated that there is thus obtained a more accurate indication of the actual phagocytic response to bacterial invasion than when serum plus washed leucocytes of another individual or species is employed.

Sodium citrate was used as an anticoagulant, since, in our experience, heparin was not so satisfactory. Two and a half cubic centimeters of blood were added to small tubes containing 0.1 c.c. of a 20 per cent citrate solution in 0.9 per cent sodium chloride. The final concentration of citrate was 0.77 per cent. The specimens were kept in the refrigerator to prevent disintegration and were

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used within four hours after they had been obtained from the patient. Specimens were thoroughly mixed just before using.

The formalized bacterial suspensions[4] were prepared by washing the growth from several twenty-four-hour-old brain agar slants with dilute formalin (0.4 c.c. of commercial formalin to 100 c.c. of a 0.9 per cent sodium chloride solution). After these suspensions of bacteria in formalin had been allowed to stand at room temperature for twenty-four hours, they were washed by centrifuging, decanting, and resuspending in fresh 0.9 per cent sodium chloride solution. This process was repeated three times. The density of the suspensions was standardized by the method of Gates,[5] so that a wire loop could be seen 2 cm. below the surface. Approximately 0.1 per cent formalin was added to the standardized suspensions as a preservative.

The phagocytic tests were set up in small, sterile agglutination tubes. The citrated whole blood (0.1 c.c.) and the formalized bacterial suspension (0.1 c.c.) were mixed by shaking the racks of tubes twenty times, after which they were placed in an incubator at 37° C. At the end of thirty minutes the tubes were removed, the contents were mixed by tapping gently twenty times, and the tubes were placed in an ice-water bath in order to check further phagocytosis. A small amount of the mixture was removed from each tube with a capillary pipette and smears were prepared as for a differential blood count. The best preparations were those on which the smear thinned out and terminated near, or just beyond, the center of the slides. It was found advisable to dry the smears rapidly by fanning at room temperature in order to avoid distortion of blood cells.

Most of the stains recommended by other workers did not appear to be satisfactory; some of them failed to stain the bacteria well, some stained the red cells too deeply, and others gave a hazy, indistinct appearance to the entire slide. Eventually, it was found that crystal violet gave excellent stains when used in the following manner: Smears were fixed momentarily in absolute methyl alcohol. While still dripping with alcohol, they were immersed in freshly prepared dilute crystal violet (1 drop of saturated alcoholic solution per cubic centimeter of distilled water) for approximately ten seconds. They were rinsed immediately in running tap water and dried rapidly in air. The bacteria stained deep purple; the white cells, lavender; and the red blood cells, bluish green.

Parenthetically, it may be noted that this method of determining opsonocytophagic power was satisfactory when used in the study of patients suffering from typhoid fever. The stained smears were examined by means of the oil-immersion lens. Most of the leucocytes were found along the sides and terminating edges of the smears. The number of phagocytized organisms in fifty polymorphonuclear leucocytes was noted and the average was then determined.

STUDY OF PATIENTS WITH ACUTE GASTRO-ENTERITIS

A number of different organisms were isolated from the stools of infants and children suffering from acute gastro-enteritis, in whose stools no members of the Shigella group were found. *Escherichia coli* were isolated from the stools of each of twelve patients; *Streptococcus faecalis* and organisms in the Aerobacter

group from approximately two-thirds of the patients; members of the Salmonella group from one-third of the patients; and members of the Proteus group from two patients. The phagocytic counts varied greatly for these organisms, and the high indices were distributed at random throughout the gastro-enteritis group and the control group without diarrhea. In none of these patients could any significance be attached to a high phagocytic index.

TABLE I

OPSONOCYTOPHAGIC INDEX ON BLOOD OF PATIENTS WITH DYSENTERY COMPARED WITH PATIENTS WITHOUT DYSENTERY USING THE SAME ORGANISM

DYSENTERY PATIENTS		NONDIARRHEA PATIENTS	
PATIENT	OPSONOCYTOPHAGIC INDEX HOMOLOGOUS CULTURE	PATIENT	OPSONOCYTOPHAGIC INDEX
D. H.	9.6	B. O.	0.3
J. H.	21.5	B. O.	0.2
R. S.	37.5		
W. W.	4.2	R. O.	0.4
R. W.	9.6*	D. A.	0.1
		J. C.	0.3
		R. E.	0.4
P. B.	9.3	R. E.	1.1
S. D.*	1.0*		
R. D.	1.4	H. M.	0.6
F. M.	27.2		
H. M.	22.8	D. P.	0.1
		F. B.	0.1

*Four weeks after onset of disease.

PATIENTS WITH DYSENTERY

If the phagocytic counts on the blood from ten patients whose stool cultures contained *Shigella paradysenteriae* are compared with those from a group of patients without dysentery, as in Table I, it is seen that the blood specimens from all the patients with dysentery tested gave increased counts with the Shigella antigens, whereas none of the blood specimens from the patients without diarrhea showed any increase. Counts above 0.5 were considered to be significantly increased. The blood was secured during the acute stage of the disease, except when otherwise noted in the tables. Variations in the opsonocytophagic index on blood of the patients with dysentery are seen in Table II, showing the tendency to increase during the acute stage and to decrease with convalescence.

TABLE II

PHAGOCYTIC STUDIES ON BLOOD OF FIVE DYSENTERY PATIENTS DURING ACUTE STAGE AND AT LATER PERIODS

PATIENT	INDEX ACUTE STAGE	TIME OF SECOND TEST	INDEX
J. H.	21.5	4 mo.	7.5
W. W.	4.2	19 days	0.2
R. W.	0.7	1 mo.	9.6
R. D.	1.3	2 mo.	0.8
H. M.	22.8	8 days	26.2
E. M.	16.3	5 days	28.1

STUDY OF A FAMILY WITH DYSENTERY

Interesting observations were made of the phagocytic counts obtained with blood from several children in one family, several members of whom had

dysentery (Table III). *Shigella paradysenteriae* of the Flexner type were isolated from the stools of four of these children, Elizabeth, Eugene, Florence, and Harriet, only the last of whom was severely ill. Two other siblings, George and Alice, also had diarrhea, but no *Shigella paradysenteriae* were isolated from their stools. The other sibling, Jennie, had no diarrhea, and no organisms of the Shigella group were found in her stools.

TABLE III

PHAGOCYTIC STUDIES IN A FAMILY IN WHOM DYSENTERY DEVELOPED

PATIENT	DIARRHEA	STOOL CULTURE	SHIG. PARADYS-ENTERIAE (HARRIET)	E. COLI (FLORENCE)	E. COLI (HARRIET)	AEROBAC-TER AEROGENES (HARRIET)	STREP. FAECALIS (FLORENCE)
Elizabeth	Yes	Positive	28.1	42.5	9.7	0.5	2.6
Eugene	Yes	Positive	16.3	16.3	4.6	0.3	1.5
Florence	Yes	Positive	8.2	37.6 (0.8*)	0.7	–	2.5
Harriet	Yes	Positive	26.2	4.5 (0.5*)	1.9 (0.6*)	0.5	1.4
Alice	Yes	Negative	28.2	29.0	7.6	0.6	1.9
George	Yes	Negative	23.4	12.5	1.9	0.6	1.4
Jennie	No	Negative	0.4	0.6	1.3	–	–
N. P.	No	–	0.1	0.8	0.3	0.2	0.7
X	No	–	0.8	4.7	–	–	–
M. S.†	No	–	2.2	23.2	0.7	0.5	1.9

*Six months later.
†Infant with scalp infection.

The four siblings who had dysentery, and the two who had diarrhea with negative stool cultures, gave high phagocytic counts with the *Shigella paradysenteriae* recovered from Harriet's stool. Jennie, who did not have a diarrhea and whose stool culture was negative, showed no increase in her phagocytic count. In the lower part of Table II it is seen that the blood of the patients without diarrhea did not have an elevated index for the *Shigella paradysenteriae*, except in the instance of M. S., an infant with furunculosis of the scalp, whose index was only slightly above normal.

SUMMARY

The whole blood from patients suffering from acute infections with members of the *Shigella paradysenteriae* group gave increased opsonocytophagic counts with antigens of these organisms. Blood from patients without diarrhea did not give increased counts with such antigens.

Blood from patients suffering from infection with *Shigella paradysenteriae* and from those with diarrhea whose stools did not contain these organisms gave high phagocytic counts at times with various bacteria other than Shigella isolated from their stools.

These tests do not furnish evidence suggesting that any particular bacteria might be the causative agent in those children suffering from diarrhea whose stools are negative for the Shigella group.

The opsonocytophagic test aids in the diagnosis of infection with *Shigella paradysenteriae*, but it is not a necessary test when it has been demonstrated that the stools of patients contain these organisms. A negative opsonocytophagic test (counts less than 0.5) probably indicates the absence of dysentery infection.

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A NEW METHOD OF STAINING WITH WRIGHT'S SOLUTION*

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THE primary requisite of a good method of staining is its capacity to reveal clearly the various structures in the blood film in order that they may be easily distinguished from one another. Simplicity, practicability, and rapidity may also be added as indispensable qualities for clinical purposes.

The method now suggested carries these features. It is as follows:

1. Cover the dry smear with a few drops of Wright's staining solution.

2. *With the breath* blow the smear as uniformly as possible until it becomes pinkish violet in color. This process will consume about ten seconds. The harder one blows, the faster will be the fixation of the stain.

3. Wash with tap water.

4. With the water running slowly over the slightly tilted slide, pour a few drops of Wright's stain. The contact between it and the smear should be made as short as possible. A long contact may decolorize the smear.

5. Wash, dry, and examine under oil immersion.

Two new features have been introduced: (1) the blowing of the smear with the breath, and (2) the washing of the stained smear with the staining solution itself. The first accelerates the process of staining, reducing its entire duration from minutes to seconds. The second dissolves the precipitated particles left in the course of staining. It thus solves one of the important objections to Wright's stain, i.e., precipitation of the solution on the slide.

Another decided advantage of this method is that in case the hematologist is not satisfied with the shade of color obtained at the first attempt, he may rectify it without much loss of time. If the smear is understained, he may repeat step 2; if overstained, step 4. It must be remembered that Wright's staining solution is a decolorizing as well as a coloring agent, depending upon the duration of its contact with the smear. This method renders the blood film

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flexible in the hands of the examiner. The process of restaining and decolorizing may be repeated several times on the same smear without the slightest injury to the cells.

If a more thorough study of the red blood cells and the platelets is desired, the following procedure may be inserted between steps 3 and 4:

3a. Dry the film, put one drop of cedarwood oil over it. Add xylol to dissolve and with the palmar surface of the small finger cover the whole slide with the solution, in the same way as when cleaning a slide after use. Dry and proceed to step 4.

This modification, which turns the film pink in color, is useful also in the examination for malarial parasites and in counterstaining after brilliant cresyl blue. It deepens the contrast between the malarial bodies, or the "net" structures and the pinkish cytoplasmic background.

I have employed this method with good results in staining smears secured from the peripheral blood and the bone marrow. I have found that it has all the advantages of Wright's and Giemsa's stains minus their objectionable features.

SUMMARY

A new method of staining with Wright's solution is proposed. It is simple, very rapid, practical, and convenient. It does not leave precipitates on the slides and shows structures very distinctly. A modification of the same for the study of the erythrocytes, platelets, reticulocytes, and malarial parasites is also recommended.

A METHOD FOR STOMACH TUBING SMALL ANIMALS*

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RECENTLY we were confronted with the problem of establishing a technique suitable for the prolonged oral treatment of a large number of rats. The procedures used by others seemed both unphysiologic and too time-consuming, and in addition entailed unnecessary risks. For example, a method necessitating the use of an anesthetic requires too much time and may have unphysiologic sequela. In our trials the use of a semirigid catheter as a stomach tube proved unsatisfactory, since the material was readily nicked by the teeth of the rat and the sharp edges of these cuts were obviously a source of irritation to the esophagus. The splendid results obtained by the method of Pugh and Tandy,[1] which employs a modified hypodermic needle, are indicative of a highly feasible method. Nevertheless, we wished to simulate more nearly the proved procedure employed for human work.

After trying these procedures with modifications, we finally developed a method which involves not only a minimal number of manipulations on the part of the operators, but also relatively little discomfort to the rat.

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During a period of six months some 200 animals of various ages have been subjected to this procedure for varying lengths of time, representing approximately 4,000 treatments without the loss of a single animal from trauma or drowning.

The procedure we have developed is easily and rapidly performed, and entails no deleterious effect to the animal.

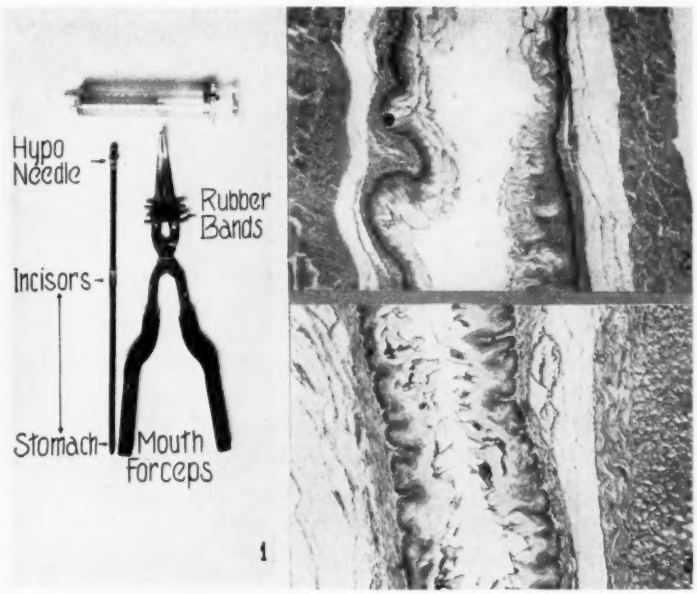

Fig. 1.—Shows the instruments used for this method.
Fig. 2.—The esophagus of an animal treated by this method three times weekly for twelve weeks (enlarged 50 times).
Fig. 3.—The esophagus of an untreated animal (enlarged 50 times).

APPARATUS

In order to open the rat's mouth and at the same time have complete control of its jaws during the procedure, the forceps shown in Fig. 1 was developed. The instrument, called a needle nose pliers, may be procured from any automobile supply store for a nominal sum. The original spring attached between the handles was considered too stiff and was, therefore, removed. To develop the necessary tension rubber bands were substituted and wound around the blades just distal to the fulcrum. In this manner the amount of tension may be nicely adjusted to please the operator.

A No. 8 or 10 soft rubber catheter was employed as a stomach tube. A piece of adhesive tape wrapped several times around the catheter not only marks the distance it is to be passed, but also protects the tube at that point from the animal's teeth.

A large No. 12 gauge hypodermic needle, which snugly fits the catheter, was used to attach it to the syringe.

PROCEDURE

The procedure requires two operators, one to hold the animal and open its jaws with the forceps, and the other to pass the stomach tube and administer the medication.

The animal is held gently but firmly, its head projecting upward between the thumb and forefinger. Excessive motion is prevented by extending the remaining fingers along the belly of the rat. With the other hand the operator inserts the blades of the forceps between the jaws just behind the incisors and applies pressure sufficient to maintain the mouth in a suitable position for passage of the stomach tube. The other operator then proceeds to pass the tube by running its tip along the roof of the mouth to gain entrance into the esophagus, and with gentle pressure pushes it downward until the strip of adhesive tape is at the level of the incisor teeth.

One of the advantages of this method is that the operator is able to feel any abnormal resistance that the tube may encounter in its course and is thus able to prevent injury or death of the animal. If this condition occurs when about two-thirds the measured distance of the tube has been inserted, one may feel reasonably certain that the trachea has been entered. If, however, the hindrance is beyond this point, it is almost certain to be at the cardiac sphincter. In either case it is best to remove the tube entirely and to repeat the process after the animal has rested several minutes.

In order to ascertain whether, after a prolonged period of treatment by this method, any unwarrantable conditions developed, each esophagus and stomach was subjected to a careful examination. Some one hundred examinations showed no gross pathology. Subsequent histologic studies revealed only one case of a traumatic ulcer. The esophagus shown in Fig. 2 is typical of an animal treated three times weekly for twelve weeks. Fig. 3 shows the esophagus of an untreated animal.

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THE MICROSEDIMENTATION RATE*

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THE usefulness of the sedimentation rate is well established in following the course of rheumatic fever and rheumatic heart disease. It has been widely studied in tuberculosis and in many other conditions. Interest in the test appears to be increasing, and the desirability of a clinically suitable micromethod would seem evident.

It is the very multiplicity of methods for the sedimentation rate that has probably kept this test from having the widespread clinical use that it deserves. Each method has required its own normal standards, and in the case of micromethods, these "normals" have often been inadequately derived, and unverified by others who have accepted them. This may explain why some authors consider that an adequate micromethod does not exist.

Recently, a monograph on sedimentation considered only venipuncture methods, and showed that the small diameter tubes of any micromethod would have a greater inaccuracy than the larger macrotubes.[4] This need not prevent the use of the micromethod clinically, although it might well interfere with the study of the nature of the sedimentation phenomenon. Uneven settling of the column of red blood cells, probably the chief criticism against the use of the narrow microtubes, has rarely occurred in my studies. (Irregular settling, whether in large or small tubes, is sometimes due to reticulocytosis, as it has been shown that the reticulated red blood cells remain at the top of such unevenly settling columns.[13])

Any method for sedimentation must provide for collection of blood with the use of an anticoagulant, adequate mixing, and suspension of the blood column in a strictly vertical position. As long as this required two to four or more pieces of glassware, and as many maneuvers, no method, micro or macro, was likely to get into widespread use in the office or clinic. Seven years ago Landau[8] published his micromethod, which was simple to the point of encouraging frequent use. I have worked with it since 1934, and wish to report certain further simplifications (but not fundamental alterations) of the method, as well as the results of my clinical studies and the normal values established with its use.

Since this is not fundamentally a new method, the results are directly comparable with previous studies.[3, 8] The results are not comparable with other methods unless the height of the blood column in the tube and the method of

*From the Adolescent Cardiac Clinic, the Medical Services, and the Cardio-Vascular Department of the Michael Reese Hospital.

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TABLE I

COMPARISON OF PREVIOUSLY DESCRIBED MICROMETHODS

AUTHOR	YEAR	NORMALS	LENGTH OF BLOOD COLUMN	BORE OF TUBE	ANTI-COAGULANT
Landau[8]	1933	1 to 8 mm. in 1 hour (174 tests on 78 children; 1 to 5 mm. in 70 men; 1 to 8 mm. in 80 women)	62.5 mm.	1.0 mm.	5% sodium citrate
Elghammer[3]	1934	3 to 8 mm. in children		Landau's method	
Cutler[2]	1927	2 to 8 mm. for men; 2 to 10 mm. for women. (Comparable to his macromethod, which also uses a 50 mm. blood column height; 5,000 patients were studied by the macromethod)	50.0 mm.	2.5 mm.	5% sodium citrate
McSweeney[9]	1934	10 mm. or less for normal children and rheumatic children in a quiescent period	100.0 mm.	1.0 mm.	3.8% sodium citrate
Beaumont and Maycock[1]	1935	0.5 to 4 PER CENT in one-half hour for men, and 1 to 8 PER CENT for women	100.0 mm.	1.13 mm.	3.8% sodium citrate
Smith[12]	1936	"Within the range usually stated by the Cutler venipuncture method for normal adults." Did 45 tests on 42 patients, but only 18 tests on patients 8 years old or over	50.0 mm.	2.5 mm.	5% sodium citrate
Rogatz[11]	1938	"All observers have found that a normal sedimentation value lies between 5 mm. in one-half hour and 10 mm. at the end of 1 hour." Reported only 7 tests on children 8 years old or over, and only 50 tests in all		Smith's method	
Vollmer[14]	1939	8 to 10 mm. in 1 hour. Over 15 mm., "marked acceleration." Did 104 tests on 52 children	44.5 mm.	2.5 mm.	3% sodium citrate
Kato[6, 7]	1940	0 to 20 PER CENT	64 to 102 mm. (Whatever the tube dimensions, a VOLUME of 50 c.mm. of blood is constant)	0.79 to 1 mm.	Special potassium oxalate formula "approaches heparin in results"

recording are the same (see Table I). The attempt to compare different methods by recording the millimeters of settling as percentages of the original blood column height is only approximately accurate, since changing the height of the original blood column does not necessarily change in like degree the observed sedimentation distance.

Landau reported his readings as *millimeters in one hour* (i.e., millimeters of fall of the top of the settling erythrocyte column). In my experience, this method is clinically adequate and preferable to other methods of reporting, such as millimeters per minute during the period of greatest fall, arbitrarily chosen index or percentage systems, or the time required to fall some given distance. A graph can be made of the rate of fall by five-, ten-, or fifteen-minute periods during the hour, but this is rarely useful, and certainly not conducive to doing many tests at one time, as in a clinic. Occasionally comparison of the thirty-minute value with the final reading appears to be useful.

The normal values previously established have been open to question. Landau's original material consisted of 78 children, admitted to the hospital for various causes, and found, after careful examination, to be without demonstrable symptoms of disease.[8] On these patients 178 tests were done. Landau adds that "we had ample experience among policlinic patients. The normal values for children over two years of age may vary from 1 to 8 mm. without any pathological condition being present. We have been unable to discover any noteworthy difference in value for different sexes with any degree of certainty. Our impression is, however, that as a general rule the normal values do not exceed 5 or 6 mm. But it is of great importance in such a phenomenon as sedimentation not to draw too narrow limits for the normal values." In 80 healthy women of the hospital staff he found values of 1 to 8 mm., and in 70 men, values of 1 to 5 mm.

The next year Elghammer,[3] using Landau's method, stated that "values of 3 to 8 mm. are to be regarded as normal rates in children; 8 to 11 constitutes the zone of possible pathologic indication, 11 to 25 a definitely increased rate, and 25 to 50 a marked acceleration."

No subsequent studies by this method have appeared in the American or English literature. Seven other micromethods have been described, each with the fault either of needless complexity of technique, or of inadequately derived normal values, or both. These are summarized in Table I, along with Landau's and Elghammer's results.

In the present study with Landau's method, about 2,600 tests were available for analysis. Experience soon showed that acceptance of previous normal standards was causing confusion and apparent unreliability. An attempt was made, therefore, to derive normal standards, statistically, and in patients clinically verified to be normal as far as activity of rheumatic infection was concerned.

It is not my purpose to discuss the nature of the phenomenon of sedimentation, influence of various anticoagulants, the detailed comparison of micromethods and macromethods, nor the question of the various so-called corrections. This has been well covered by others.[4, 5, 15] Landau's method has been found useful clinically without considering any corrections other than that of room temperature. This should be between 60° and 80° F.; if the room is 5° to 10° F. above this, the reading may be 2 to 4 mm. higher; if the room is correspondingly below 60° F., the reading may be 2 to 4 mm. lower than the "true" reading (see also Appendix at end of paper). Fortunately, the present trend of

investigators is to realize that "corrections" may well favor incorrect clinical judgments. Hambleton and Christianson,[5] and Kato,[6] have stressed this in particular.

Of the 2,600 tests mentioned, which were the basis of this study, 2,094 tests were made on patients, from 8 to 23 years old, with cardiac involvement. The immediate purpose was to establish "normal values" for this group, so as to make of the test a useful indicator for judging the presence or absence of *active* rheumatic infection. The following were the criteria for selecting tests for statistical analysis of the normal range:

1. Absence of any clinical evidence of *active* rheumatic infection or carditis at the time of the test.

2. Absence of any type of infection or recognized disease, other than the inactive rheumatic fever, at the time of the test.

3. Absence of any suspicion of such rheumatic activity or other disease or illness within a month before and after the given test.

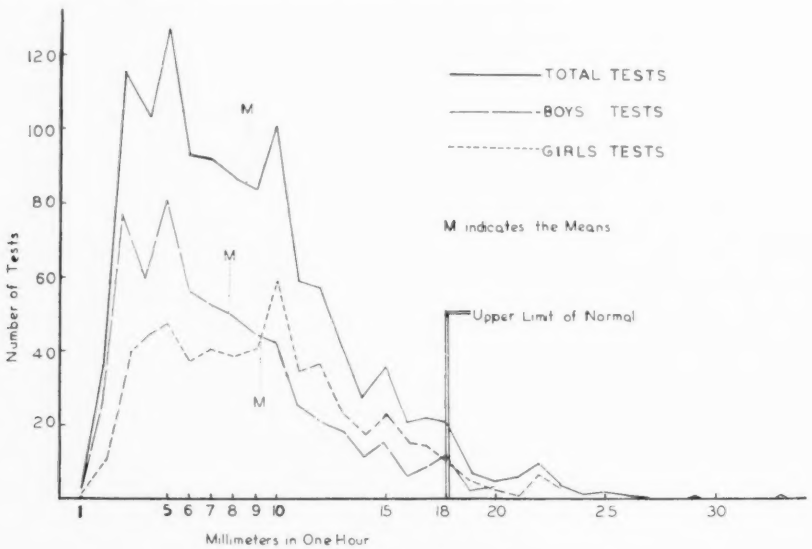

Fig. 1.—Distribution curve for determining normal values.

Tests not meeting these criteria were not included as a "normal" for statistical study even if they fell unquestionably within the normal range.

On the above basis, 1,165 tests were suitable for analysis, 620 on boys and 545 on girls. Of these, 352 were done personally; the remainder were done at the Sunset Camp for Cardiac Children by eight resident physicians under my supervision.* In many instances, the children were seen year after year, at the camp and in the various clinics in Chicago.

Fig. 1 shows the distribution curve of these 1,165 tests. It is seen to be skewed to the left, but it is reasonable to accept it as being within the limits of a normal distribution curve. In such a case the data from which the curve

*A special statistical study was made to demonstrate the fact that these residents learned the method quickly, and that their results were uniformly comparable and reliable.

is constructed may be used for the statistical derivation of a range of normal values. The basis for these statements is found in Table II, as well as the results of the statistical study. In a series of studies, one of the purposes of which was to establish normal values for a modified macrosedimentation method, Osgood and his associates[10] have shown that their observed values for the sedimentation rate also follow a skew curve.

TABLE II

RESULTS OF A STATISTICAL ANALYSIS OF 1,165 MICROSEDIMENTATION TESTS FOR THE DETERMINATION OF NORMAL VALUES

	TOTAL	BOYS	GIRLS
Number of tests	1165	620	545
Range	1 to 33	1 to 33	1 to 27
Mean	8.52	7.80	9.33
Mode	5	5	10
Standard deviation	4.62	4.67	4.57
Average deviation	3.54	3.47	3.63
Standard deviation divided by average deviation*	1.31	1.34	1.26
Error of mean	±0.14	±0.19	±0.20
Upper limit of normal	17.76	17.14	18.47
Upper limit of normal for practical purposes	18	18	18

*For a "normal curve," this quotient is usually considered to be 1.25 or less. The values here shown are trivially over the limit.

Inspection of Fig. 1 shows a range of one to 33 mm. in one hour. However, the upper limit of normal is not to be taken as 33 mm. The mean is 8.52 mm., and as seen in Table II, the standard deviation of the mean for all tests is 4.62 mm., with the standard error of the mean 0.19±. In other words, the mean really is somewhere between 8.33 mm. and 8.71 mm., and the average variation from the mean of any given test is 4.62 mm.

For practical purposes the range of normal values is usually considered to be two standard deviations in each direction from the mean, when such data is available and forms a curve whose degree of skewness is within the limits of a normal distribution curve. Hence, 2 times 4.62 (the two standard deviations) plus 8.52 (the mean) equals 17.76 mm. Since the test is read only to the nearest millimeter, it may be stated that 18 mm. in one hour is the upper limit of normal for all tests.

The lower limit becomes less than zero, which would mean, statistically, that there was no such thing as too slow a rate. Actually, this is not the case, as will be discussed subsequently.

Theoretically, 95 per cent of all the tests are included in this total range of four standard deviations. Actually it here includes 97.5 per cent. Although the half dozen or so "unexplained" high values are almost certainly artifacts, they are not excluded from analysis.

Reference to Table II again will show that the upper normal limit for girls is slightly higher than for boys, and this is true also of the mean values. These differences can be shown to be statistically significant, but practically they are unimportant, being scarcely more than a millimeter. As stated previously, the test is read only to the nearest millimeter.

Twenty-five per cent of these tests are higher than 10 mm., the previously accepted normal limit. In reviewing the records of *all* those patients in the 11 to 18 mm. group, it is found that, first, many patients with mild upper respiratory infections and various minor conditions have normal rates, that is, 18 mm. or less. It has been an aid to clinical thinking to regard these as normal rather than as "slightly elevated" rates.

Secondly, and more important, no one with a rate consistently 18 mm. or less had *active* rheumatic fever or carditis at the time of the tests. Seeming contradictions to this are rare. The most important exception is congestive heart failure following acute rheumatic carditis, when the rate may fall to normal levels (perhaps because of liver damage with consequent decrease in blood fibrinogen).

No further comment is necessary about the well-recognized high rates in the presence of acute rheumatic fever or carditis, nor the fact that the high rate may have some other explanation.

The finding of a rate of 1 mm. or less should suggest first a clot in the tube. In its absence, the possibilities of polycythemia, liver damage, and finally, an allergic state should be considered. I have observed for some years an adolescent girl with mitral stenosis and true bronchial asthma whose sedimentation is usually 4 to 10 mm. When she developed a mild acute polyarthritis and carditis, her rate rose to 16 mm., regarded as abnormal for her.

There is a great value to repeated tests on each patient, so that the normal or control values may be established. In this way the significance of changes may be better appreciated. Normally, the sedimentation rate does not change more than a millimeter or two from day to day or week to week. Furthermore, there are no variations after eating, exercise, or bathing, such as cause confusion at times in leucocyte counts.

Serial rates are especially important in judging control values in middle-aged and elderly patients, in whom the diagnosis of "normal health" is always an increasingly difficult matter, and normal standards for sedimentation are harder to define. Approximately an additional 500 tests were studied in these older patients. For the present it has been found useful to apply the same normals as for the younger patients.

In the case of the rheumatic patients, the most difficult decisions as to management often come in that group with clinically borderline or questionable activity of infection. Herein the test has had its greatest practical usefulness. I have not been led astray by using this higher upper normal limit. It has served as an aid in deciding that in some cases 99.2° F. was to be regarded as the normal mouth temperature; in others that a heart rate of 100 did not depend on active carditis; in still others that loss of weight and appetite were not indications of recurrent rheumatic infection.

It should be restated that these comments are valid *at the time of the test.* In fact, the finding of a normal rate aids in interpreting the events of the past week or weeks rather than in foretelling the future. A normal rate may rise to 35 or more several days after the onset of an acute infection. In my experience

an equally rapid fall from high levels has not been encountered; several weeks or more are required after the acute episode is clinically over; indeed, sometimes it is several months.

Conversely, the finding of an abnormally high rate must be taken seriously, and an effort should always be made to find the reason for it. Increased sedimentation is not usually the earliest evidence of active rheumatic infection, but it may be, especially in the case of recurrences. Other significant signs or symptoms will appear in a week or two, rarely as long as a month. A preceding upper respiratory infection may have been responsible for this apparent "earliest evidence," but all upper respiratory infections are not followed by rheumatic activity; hence the rate may return gradually to normal. However, rheumatic reactivations are by no means all preceded by upper respiratory infections, so that the clinical reason for the early increase of sedimentation (before fever or tachycardia appears) may not be readily explainable.

SUMMARY

In a study of nearly 2,100 microsedimentation tests by Landau's method on 405 patients with cardiac disease, ranging in age from 8 to 23 years, values have been derived which are normal for inactive rheumatic infection, and probably for the age group as well. The upper limit of normal is 18 mm. in one hour, although the mean is 8.5 mm., and 75 per cent of the tests are 10 mm. or less. The results fall within the limits of a normal skew curve, permitting statistical analysis.

The Landau micromethod is advocated because of its simplicity and the absence of need for applying corrections.

No contradictions have been encountered to the rule that a rate of 18 mm. or less eliminates active rheumatic infection or carditis at the time of the test, with rare and fairly well understood exceptions, the most important of which is congestive heart failure following acute carditis. Conversely, a high rate must always be taken seriously, even in the absence of other signs or symptoms, and an effort must be made to find the reason for the high rate.

The possible significance of very low rates is discussed.

Approximately an additional 500 tests were studied in patients without rheumatism and in middle and elderly age groups; in the latter the diagnosis of "normal health" is always an increasingly difficult matter, and normal standards for sedimentation are harder to define. For the present, it is found useful to apply the same normals as for the younger patients, with emphasis on the establishing of control values by serial testing of each patient.

APPENDIX

Technique for Sedimentation Test.—Prepare the finger as for a blood count; for this acetone is preferred. Insert the short end of the pipette into the rubber tip of the aspirating syringe. After the patient's finger has been stuck, draw 5 per cent sodium citrate solution into the tube to the lower mark (12.5 mm. from the tip), wipe off the excess from the outside, and insert the tip into the drop of blood on the finger. (The first, second, or third drop may be used, with or without squeezing; if the coagulation time happens to be rapid, the fourth drop may cause error.)

Draw the blood into the syringe by slowly unscrewing the plunger of the syringe; the blood mixes with the citrate as it is drawn up, and when the advancing column of fluid, regardless of whether it is blood, citrate, or blood-citrate mixture, reaches the second mark (50 mm. above the first mark), no more blood is drawn into the tube. Remove the pipette from the finger, draw the fluid column up several millimeters more, and then wipe off the tip of the pipette. It is easy and desirable to develop the practice of holding the assembled outfit of pipette and syringe in one hand during this phase of the procedure, during which the tube should be held nearly vertically.

Now mix the blood by drawing it into the bulb of the pipette so as to leave about 5 mm. still in the straight part of the tube, and then re-expel it. This is continued until the blood has been drawn into the bulb and expelled at least eight times. After the fourth time the tube may be rotated 180° about its long axis for subsequent mixings. During this time it is well to hold the tip of the assembled outfit somewhat lower than the syringe while the blood is being drawn into the bulb and re-expelled. This tends to prevent the formation of air bubbles in the blood. However, the fluidity of the blood is sometimes such that the tube must be kept nearly vertical at all times; otherwise the blood will be broken up in the bulb by air bubbles.

It is important to avoid handling the bulb with the fingers; to do so warms the air in the bulb. When the tube is finally set up at room temperature, the cooling of the bulb may draw the blood column partly back into the bulb. If this occurs, remixing is necessary. Occasionally one can manipulate the outfit to expel an air bubble which may have formed by holding the tip uppermost. If the blood column is intact for all but a millimeter or two at one end after final mixing, significant error does not result. Otherwise, or if more than one air bubble has appeared, the test may be "salvaged" by expelling all the blood into a small depression such as that of a hanging-drop glass slide, and then drawing it back slowly so as to exclude the bubbles. I wish to emphasize that in practice this salvaging maneuver is rarely necessary.

After these mixings, when the blood is in the straight part or bore of the tube, put a finger of the left hand tightly over the tip of the pipette, and slowly remove the syringe with an even, unscrewing motion. Remove the left finger and press a small ball of plasticine firmly against the upper end from which the syringe has just been removed. Turn the tube upright quickly and place it in the wooden block. This maneuver requires no more than twenty seconds after the mixing is stopped, and if as much as sixty seconds elapses, an error may be introduced. The time for setting up a test, from start to finish, need not be over one and one-half to two minutes.

When the blood is let into the bore of the tube for the final time, the apparatus is turned into a vertical position, and the blood is slowly expelled from the bulb, so that a minimal amount adheres to the walls of the bulb. The bottom of the column should be about 15 mm. from the tip—the exact point is immaterial. An accurate millimeter rule is used to measure the distance of settling.

One of the outstanding advantages of this technique is that the test can be set up at some later time (up to six hours) and can be readily transported, e.g., from patient's home to the office. In this event, after mixing and putting on the plasticine seal, one may carry the tube horizontally or nearly so. (A fountain pen box makes a useful container in the bag.) When it is subsequently to be set up, the following is the procedure:

Hold the tube horizontally. Remove the plasticine by drawing the fingers toward it along the outside of the tube from the bulb to the upper end; in this way, it is removed without squeezing any of the plasticine into the lumen. If the plasticine is unduly soft, the tube may be plugged in spite of precautions. In such a case, a fine wire may be inserted into the plugged end and the plasticine broken up sufficiently to permit use of the screw-suction syringe. This is an infrequent "complication."

When the plasticine is off, hold the tube horizontal, put a finger of the left hand firmly against the tip of the tube, and hold it there while the syringe is put on with an even screwing motion. There is now unequal pressure on the two ends of the blood column, which must be equalized before mixing. With the left finger still applied, unscrew the plunger of the

syringe about three-fourths turn, and remove the left finger cautiously, as though handling a volumetric pipette. If the blood column moves toward the tip, a little further unscrewing of the plunger is required, perhaps another one-quarter turn. If the blood column advances into the bulb, the plunger must be screwed in slightly. A little practice is required to do this without admixture of air bubbles or loss of blood, but the knack is quickly acquired.

Then proceed to mix as before, with this very important difference, that more mixing is required when the tube has been transported as already described. I have determined experimentally that 20 mixings in the bulb are not enough, and that 24 are usually sufficient. To be sure, then, mix the blood 32 times, eight times in each of the four quadrants of rotation about the long axis of the tube. This may sound like too many times, but it is accomplished in one and one-half minutes or less. When thus mixed, the tube is set in the wooden block, as previously described. I wish to stress again that the tube must be strictly vertical, and at a room temperature of 60° to 80° F. (A room temperature of 90° F. adds 2 to 4 mm. to the reading; the error is not constant, and depends upon what the original reading would have been; temperatures below 60° F. retard the fall by a like amount.) Furthermore, if the room temperature changes suddenly, for example, from 75° to 65° F. in a few minutes, the entire blood column will move in the tube. Error is thereby introduced, as settling cells tend to become mixed with the already clear plasma above the line of demarcation.

Materials.—Tubes of the Landau type and the "Gomco" screw-suction aspirating syringe are obtainable from commercial supply houses.*

A wooden block, approximately 2½ inches by 1 by ¾ inch, with holes machine-drilled in it so as to have them strictly vertical, will accommodate the tubes. Eight or ten holes can be drilled in such a block by any machine shop or carpenter. Most of the holes should be drilled with a No. 12 standard bit. To take care of chance odd sizes in the external diameter of the tubes, two holes can be drilled with a No. 11 bit, and two with a No. 13. A thin brass plate on the bottom will prevent uneven wear which may tend to throw the holes out of vertical alignment. (It has been shown that as little as 2 per cent deviation from the vertical will accelerate the settling in the tube, and thereby introduce error.[15]) A small pocket-sized spirit level may be used to check the levelness of the table or whatever support is used for the block.

A small package of plasticine or modeling clay, and 5 per cent sodium citrate solution, complete the outfit.

The somewhat more elaborate apparatus originally described by Landau was imported, and so far as is known is not now obtainable because of war conditions. I have used the original outfit, but I find it no more accurate, and indeed, less convenient. It was, furthermore, very expensive, which is not the case with the assembled apparatus herein described.

Acknowledgment is made to the Sunset Camp for Cardiac Children for studies made there.

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SIMPLE, SHIELDED BIPOLAR ELECTRODES*

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IN 1933 one of us (B. C.), working in this Laboratory, described a method for stimulating autonomic nerves in the unanesthetized animal by means of implanted electrodes.[1] Because of their flexibility these electrodes were able to withstand the strain of being imbedded in moving parts for a considerable period of time, and, because soft rubber was used throughout, there was no injury to the nerve or adjacent tissue. During the intervening years a bipolar modification of these electrodes has been developed for use in acute experiments. They can be easily and inexpensively made, and are adaptable to nerves of various diameters and situations. In constructing the electrodes, which are shown diagrammatically in Fig. 1, the following directions should be followed:

1. Attach eight 10 feet lengths of No. 32 enameled copper wire to the end of the shaft of a motor and revolve the shaft until the wire is evenly and closely twisted. The result is a very flexible conductor. This will be called the "flexible wire" (*F*). It should be cut into lengths of one foot.

2. Make loops (*A*) of soft silver wire (No. 27), about 2 to 3 mm. in diameter, to fit inside a No. 12 French catheter, a size generally useful. Leave about 1 cm. of the wire extending from the loop; to this extension wind and solder one end of the flexible wire (*F*) from which the enamel has been scraped. This soldering should be done before the loop is put into the catheter, otherwise the rubber will be damaged by the heat.

3. Cut a length of the catheter slightly greater than the desired distance between the electrodes and split it longitudinally. Make two holes (*B, B*) with a cutting needle, each one 2 mm. from the end of the tube and 2 mm. from the split edge. Carefully insert through the holes the silver loops and tie each of them in place with heavy silk (*C, C'*). Coat each wire, near its emergence from the catheter, with a good quality of rubber cement, and then slip over it a 3-inch

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piece of fine white stitch tubing (*D*, bore 1/32, wall 1/64 inch) pushing it firmly against the catheter. In the figure this tubing is not shown covering the nearer wire.

4. After the two emergent wires have been thus prepared and covered with the stitch tubing, tie them together in the covered portion. Coat the tubing and the outer surface of the split piece of catheter with rubber cement. Beginning with the free edge of the piece of catheter, near *C'C'*, wrap over it a strip of finest dental rubber dam, and continue the wrapping around the stitch tubing. Thus the end connections are insulated and short circuits are rendered impossible.

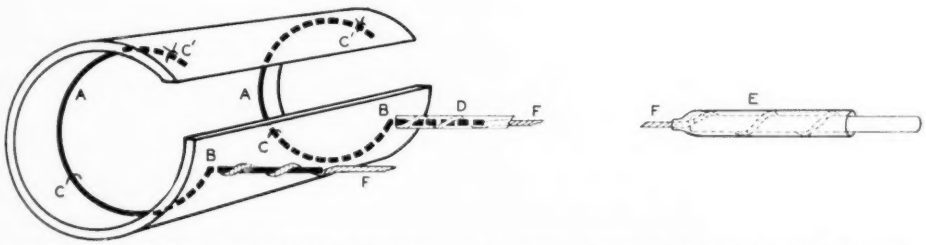

Fig. 1.—Diagram of shielded electrodes. *A*, *A*, silver wires; *B*, *B*, holes in the catheter; *C*, *C*, silk ties to hold silver wire loops in place; *D*, rubber tube covering junction of silver wire and twisted flexible wire (*F*) (tube not represented over nearer junction); *E*, rubber tube covering junction of solid wire with twisted flexible wire (*F*).

5. Slip over each flexible wire (*F*) a 1-inch length of very small bore gray rubber tubing. To the scraped free ends of these wires solder a short piece of solid copper wire and then slide the rubber tubing back to cover the junction (*E*). This solid wire will serve as a connection to an induction coil.

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TECHNICAL FACTORS INFLUENCING THE WELTMANN SERUM COAGULATION REACTION*

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IN 1930 Weltmann described a serum coagulation reaction taking place in dilute calcium chloride solution, which he used as the basis of a nonspecific laboratory test for the presence of certain general pathologic processes. The test has been accepted widely in Europe and to an increasing extent in this country. There have been numerous reports of its value in following the course and therapy of various diseases, including rheumatic fever. Comparisons between the serum coagulation reaction and the sedimentation rate as guides to the treatment of the rheumatic fever patient have been made, and it was such a problem that first attracted our attention. While performing many Weltmann tests certain technical sources of error were found, and since these have not been mentioned previously, we wish to report them, together with means for their elimination.

The method of testing serum coagulability has been well described by Weltmann[1] and Teufl,[2] and their directions were followed in performing and interpreting the reactions. Accordingly, we made up a stock solution of calcium chloride anhydrous, reagent Merck, a form of the salt containing at least 96 per cent calcium chloride. This solution was diluted further with distilled water to the concentrations necessary for the reaction. We then tested several normal sera with these dilutions but found that instead of a normal coagulation band (C. B.) of 6 or 6.5, they all gave bands of 8 and 9, or even 10. All workers agree that a coagulation band greater than 6.5 or rarely 7 indicates the presence of a proliferative or fibrotic process, while one less than 6 indicates an inflammatory or necrotic lesion. Triple distillation of the water failed to correct this, and in order to have less calcium chloride in our solutions we deviated from the directions by using calcium chloride dihydrate, USP XI, which contained 75.49 per cent of the anhydrous salt. Again we found the coagulation band of normal persons to be 7 or 8, and occasionally 9. In order to have even weaker solutions we then employed calcium chloride hexahydrate, reagent Merck, as described in a later paper by Weltmann and Sieder,[3] and a recent one by Levinson and Klein.[4] Of this preparation only 50.66 per cent is the pure salt. Even these weaker solutions produced coagulation bands of 7, 8, and 9 when normal sera were used. We never secured a normal coagulation band of 6 with any of these calcium chloride solutions.

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Testing the acidity of the electrolytic solutions by the colorimetric method, we found them to be between pH 6.3 and 6.5, which was the same as that of our distilled water. Because Weltmann[1] had said that his solutions were alkaline, and Levinson and Klein[4] had found theirs to be between pH 7.5 and 7.73, we adjusted ours to 7.6, with 0.1 normal sodium hydroxide. This was done by adjusting the distilled water to pH 7.6 prior to preparing the solutions to avoid their subsequent dilution. This was not a significant addition of cations, since Weltmann[1] found that the monovalent sodium did not react in the same way as the bivalent calcium. With our solutions adjusted in this way we tested a large number of normal sera and found each of them to have a coagulation band of 6 or 6.5.

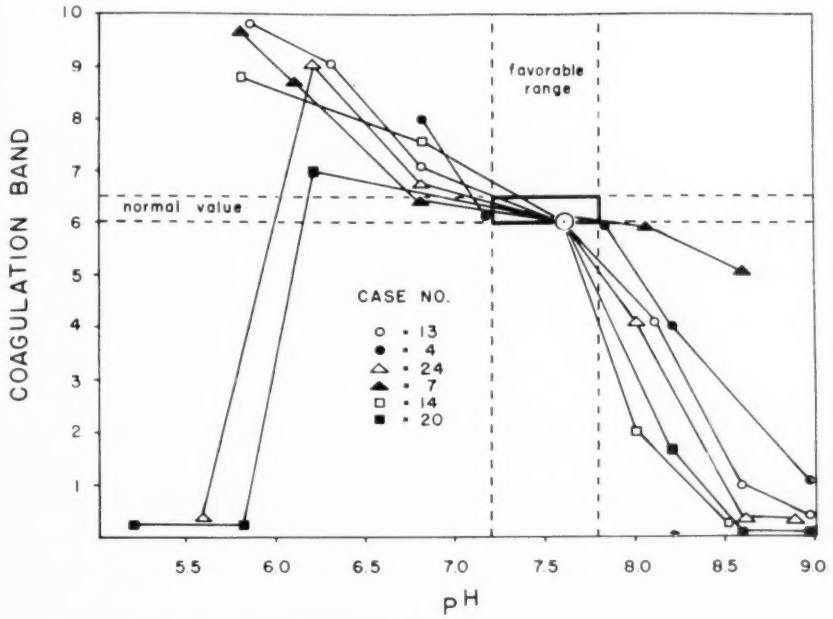

Fig. 1.—Behavior of coagulation bands of six normal sera at different pH values.

Accordingly, we studied in more detail the influence of the pH upon the coagulation of the serum. We found that as the solutions became more acid, the coagulation band was higher or shifted to the right; and as they became more alkaline, it was decreased or shifted to the left. When this phenomena was examined more closely, we found all normal sera reacted similarly in the pH range from 7.2 to 7.8, by giving a coagulation band of 6, or rarely 6.5. In more acid or more alkaline calcium chloride solutions normal sera did not always give the same coagulation band; for example, at pH 8.6 normal sera had coagulation bands from 0 to 3, and at pH 5.6 most coagulation bands ranged from 8 to 10. In some sera, however, the coagulation band would first shift to the right as the testing solutions became more acid; but when a pH of about 5.8 or less was reached, the coagulation band became 0. In these cases no observable change took place in the tubes, not even a clouding of the fluid. This led us to believe there probably had been some change in the coagulable protein. These effects of the pH were

found regardless of the strength of the calcium chloride salt used to prepare our testing solutions. For instance, with the dihydrate of the salt, a pH of 8.4 to 9.0 was necessary before normal serum gave a coagulation band of 6, and in less alkaline solutions the band was higher. Fig. 1 records the coagulation band of 6 normal sera tested with calcium chloride solutions of different acidity and alkalinity. Many others tested gave similar results.

The only references to the hydrogen-ion concentration in connection with the Weltmann test, which we could find, were those of Weltmann,[1] Levinson and Klein,[4] and Dees.[5] Weltmann[1] said that his solutions were alkaline; this may have been due to the alkalinity of the water in Vienna, a fact known to one of us (F. S.) during previous work. Levinson and Klein[4] mentioned the pH of their solutions but believed it had no effect on the coagulation band. In the same paper they report the influence of the serum pH on the reaction, pointing out that a decrease of the pH caused the coagulation band to shift to the right, while an increase caused the reverse to take place. This fact became more evident when their studies were repeated on the same patient. Despite this, they believed that there was no essential relationship between the pH and the coagulation band. Since we found the alkalinity of the testing solutions influenced the coagulation band, we felt that because the serum was diluted fifty times by these solutions, the pH of the mixture would be more nearly that of the larger volume of fluid. In other words, the pH of 5 c.c. of these solutions was not changed enough by the addition of 0.1 c.c. of serum to influence the reaction.

TABLE I

	Amount $CaCl_2$ Solution c.c.	*pH $CaCl_2$ Solution*	*Amount Serum c.c.*	*pH Serum*	*pH Mixture*
1.	2.5	9.0	0.05	7.6	8.6
2.	2.5	8.6	0.05	7.6	8.4
3.	2.5	8.2	0.05	7.6	7.9
4.	2.5	7.6	0.05	7.6	7.6
5.	2.5	6.2	0.05	7.6	6.8
6.	2.5	5.8	0.05	7.6	6.3
7.	2.5	5.2	0.05	7.6	5.8
	Amount $CaCl_2$ Solution c.c.	*pH $CaCl_2$ Solution*	*Distilled H_2O c.c.*	*pH H_2O*	*pH Mixture*
1.	5	8.4	0.1	7.6	8.3
2.	5	8.0	0.1	7.6	7.9
3.	5	7.6	0.1	7.6	7.6
4.	5	7.0	0.1	7.6	7.1
5.	5	6.7	0.1	7.6	6.8
6.	5	6.1	0.1	7.6	6.2
7.	5	5.6	0.1	7.6	5.8

Dees[5] found that the pH of the calcium chloride solutions was variable and ranged between 5.25 and 6.30, but that when the serum was added the pH of the mixture became 7.2 to 7.4 in every case. She concluded that this was due to the buffering action of the serum, and that, for this reason, the acidity of the testing solutions was unimportant. In an attempt to repeat this, we adjusted the pH of our solutions and tested the influence of the serum on them at the various adjusted values, but failed to secure a strong buffering effect. That

there is slight buffering action is shown when water of the pH of the serum is added to the testing solutions in the same amount as serum, but this is not strong enough to bring the mixture to the favorable range if the solutions are very acid or alkaline. Many sera were tested in this way, and examples of our findings are shown in tabular form in Table I.

An incidental finding was that if our solutions were exposed to the air for any length of time before the test was made they became more acid, and consequently the bands shifted to the right. This was probably because of absorption of carbon dioxide from the atmosphere. Probably for the same reason the reagents became more acid daily, and, for this reason, the pH was tested and if necessary readjusted each time they were used. Because of this change we found it advisable to test a serum of known coagulation band when pathologic sera were tested, as well as whenever fresh solutions were made. This control need not be human serum but may be normal rabbit or sheep serum, which usually have a coagulation band of 6, but occasionally 5 or 7. In this connection it should be mentioned that Hennes and Kemen[6] advise making up fresh solutions every time the test is performed. This may have been due to the same unfavorable factors that influenced our reactions.

TABLE II

Material	*Coagulation Band*
Normal beaten rabbit plasma	5
Normal beaten rabbit plasma + oxalate	5
Normal human serum	6
Normal human serum + oxalate	6

Weltmann,[1] Teufl,[2] and Kaiser[7] always used fresh serum, and Levinson[8] said it was best to use fresh serum, but that it might be stored for as long as four days in the cold. We found that serum which was kept sterile and refrigerated gave the same coagulation band weekly for four and five weeks' time. Knowledge of this was valuable if for any reason we wanted to retest a serum.

We found that the reading of the test should be made immediately after the tubes were removed from the boiling water bath. If they were allowed to stand for any length of time, tubes which were densely cloudy without coagulation when removed from the bath, later showed a precipitate which might have been confused with coagulation. Hence tubes which should have been called negative would then be read falsely as positive, the coagulation band would be shifted to the right, and be greater than if the tubes were read at once.

Weltmann and Medvei[9] found that when they tested plasma of blood defibrinated by agitation with glass beads, the coagulation band was the same as that of the serum. In our experience plasma of blood treated in this way had a coagulation band that was shifted one or two tubes to the right of the serum value. It must be considered, however, that plasma treated in this way is slightly hemolytic, even though the agitation with the beads is done as carefully and as gently as possible. It is generally known that hemolysis increases the coagulation band. Plasma of blood treated with a mixture of potassium and ammonium oxalate usually had a coagulation band one or two less than the serum. Occa-

sionally it was the same as the serum, and very rarely it was slightly higher. That this was not due to the addition of oxalate (the mixture which has been used contains 3 Gm. of ammonium oxalate and 2 Gm. of potassium oxalate in 100 c.c. of distilled water; 0.08 c.c. has been used for 2 c.c. of serum) could be seen when oxalate was added to serum or beaten plasma, and the mixture was tested one hour later showing no change in the coagulation band (Table II).

SUMMARY AND CONCLUSIONS

The importance of using the hexahydrate of calcium chloride for solutions employed in the Weltmann test has been re-emphasized. The pH of these solutions was found to be an important factor influencing serum coagulation, and the favorable range for this coagulation is between pH 7.2 and 7.8, with an optimum value of pH 7.6. Since the buffering action of the serum used in the test is not great enough to secure favorable values, the reagents must be adjusted to the suggested range.

If such adjustment is made, the pH of the solutions should be determined every time before use, and a control serum of known value should be tested with unknown pathologic ones. This may be the serum of a suitable laboratory animal.

Serum tested need not be fresh if it has been kept sterile and refrigerated after collection.

The test should be read immediately after the tubes are removed from the boiling water bath to avoid a false positive interpretation.

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AUSCULTATORY BLOOD PRESSURE METHODS FOR DOGS*

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THE best blood pressure method accepted today for hypertension experiments in dogs seems to be that of subcutaneous arterial puncture,[1] which gives accurate pressures with little disturbance of the animal, and in expert hands permits fairly frequent repetition. However, when it is desired to make numerous daily estimations continued during many days or weeks, some indirect method is necessary. Aside from the antiquated van Leersum carotid loop, the choice is between oscillometry, palpation, and auscultation. The need for comprehensive serial pressure readings prompted me[2] to devise the first auscultatory method for dogs as one of the preparatory steps for the contemplated renal-vascular research in 1923. The consensus of trials by various workers[3] has credited it with a reasonable degree of accuracy. Assuming, for argument, that errors may occur even greater than those reported, possibly to the extent of 10 or 15 mm. deviation from the true intra-arterial pressure, the general conclusion remains that direct methods may be used for precise acute experiments or for occasional checks on the indirect readings. For chronic experiments the frequently repeated readings by the convenient auscultatory method give a better picture of the general condition than a smaller number of technically exact determinations, which are subject to the far greater variations in the animal from hour to hour or from day to day. The reasons which make auscultation the preferred method for man may hold also for the dog.

Valuable criticisms and suggestions were made by Ferris and Hynes[4] in 1931. Upon finding an opportunity to resume this work recently, I have attempted a further improvement, which is illustrated in Fig. 1. The dog with very little training learns to lie quietly on his right side, and the pressure is taken in the left thigh. The hair is preferably, but not necessarily, clipped. The adapter, molded in hot water from dental gutta percha, is slightly curved to fit the posterior margin of the thigh. This appears to be an advantageous innovation as compared with former devices fitted to the external surface, because there is so little variation in the posterior margin that the one simple adapter will serve for most kinds and sizes of dogs. The adapter is thin above and thick below to compensate for the taper of the thigh. The surface is slightly roughened, and there are flanges above and below between which a 4 inch cuff can be held securely. Instead of a harness attachment to the body, a cord passes to a weight hanging over the edge of the table, heavy enough to assure that the adapter and cuff cannot slip downward.

Readings on the femoral artery are taken by means of a stethoscope with a flat bell slipped slightly under the lower margin of the cuff. There is a

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Fig. 1.—Adapter in position on posterior margin of left thigh, ready for application of cuff.

Fig. 2.—Dog lying on right side; left foreleg grasped with forefinger above olecranon; cuff applied above it.

possible advantage if the thick lower edge of the adapter spares the stethoscope slightly from too tight pressure under the cuff. If the bell piece is inserted too high, fictitiously high readings are obtained, just as when too narrow a cuff is used. Some practice is needed for the best wrapping of the cuff and accuracy of readings. Fairly large dogs are preferred, but readings have been obtained in Scotch and fox terriers. The feasibility of smaller equipment for still smaller dogs has not been investigated.

Sounds are audible in surprisingly small arteries, and I have confirmed the observation of Corcoran and Page[5] that in some large dogs readings are possible in the dorsalis pedis with a 2 inch cuff above the ankle. But, since attempts have been made for several generations to find a satisfactory method for the cylindrical foreleg of the dog, it was a surprise to learn finally that brachial artery readings are practically as simple and as distinct as in man. As shown in Fig. 2, the animal's left elbow is held straight by the left hand with the forefinger keeping a continuous grip above the olecranon. A 2 inch cuff is applied around the humerus above this forefinger. The cuff is thus kept high enough until the stethoscope is applied below (outside) it, at or slightly above the elbow joint. The difference from the femoral readings is similar to that between the human arm and thigh. Both systolic and diastolic sounds are usually so sharp and clear that this method may be favored over the more complicated femoral method. The systolic fluctuations are not as wide as in the femoral. In both, the diastolic may resemble that in man, or there may be either low or high readings of which the cause or significance is unknown. The method for both limbs, however, is quick and easy, and each can serve as a check on the other.*

Shivering, panting, etc. may interfere seriously. Satisfactory sounds may be unobtainable in some states of anesthesia, hypotension, or weakness. The method, therefore, is not adapted to all types of experiments. For our hypertension studies, however, the numerous convenient combinations of systolic and diastolic, femoral and brachial readings have appeared more valuable than occasional mean pressures in a single artery.

SUMMARY

Convenient auscultatory methods are described for estimations of femoral and brachial blood pressures in dogs. Some advantages and limitations for different types of experiments are indicated.

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THE LAUGHLEN TEST FOR SYPHILIS*

An Evaluation Based on Comparison With 3,100 Hinton Tests

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In 1935 Laughlen[1] described a flocculation test for syphilis which employs a special modification of the Kahn antigen. Among the advantages claimed for this test were simplicity of performance, speed, accuracy, and ease of reading the results. Essentially, the antigen for the Laughlen test is prepared from the Kahn antigen by the addition of cholesterol, scarlet R dye, and balsam. The dye, which is insoluble in water, colors the lipoid particles of the antigen but does not color the liquid in which they are suspended. Thus, when flocculation occurs after mixing a drop of antigen with a drop of the serum to be tested on a glass slide, the clumped particles become visible to the naked eye and the reaction is, theoretically, easy to read. The directions for the preparation of the Laughlen antigen are outlined in the original report. Preparation of the antigen is somewhat complicated and considerable experience is necessary to adjust it to the proper sensitivity. Since 1937, however, a uniform and stable "Laughlen reagent," in its inactive form, has been produced by the Lederle Laboratories, and the antigen for the tests reported here was obtained from that source. Activated antigen for performing the test is prepared from the inactive material by the addition of 10 per cent sodium chloride. The exact amount of 10 per cent sodium chloride necessary to activate 1 c.c. is marked on each lot of antigen. Inactive antigen is stable for approximately six weeks, but the activated Laughlen reagent will keep for seven days only, after which it becomes supersensitive. The antigen must be activated twenty-four hours prior to its use; it has been our practice to activate antigen once a week in a quantity sufficient to last seven days so that it is always available for use. Ordinarily, 1 c.c. of antigen suffices for about 75 tests.

In his first communication Laughlen,[1] reporting on 400 routine blood specimens, found that his test showed 99 per cent agreement with the Kahn test, and 98 per cent agreement with the Wassermann test. These findings were

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confirmed in general by Robinson and Stroud,[2] and Price.[3] The former investigators, in 1,000 routine tests on hospital patients, found 97 per cent agreement with the Kahn test and 93 per cent agreement with the Wassermann test. Later, Laughlen[4] studied more than 5,000 specimens according to his method and reported an agreement of 98 per cent with the Wassermann test, and 99.4 per cent with the Kahn test.

Originally, Laughlen[1] stated that his test could be performed successfully on spinal fluid as well as on fresh noninactivated blood samples. It is now generally agreed, however, that this is not so, and the pamphlet which accompanies the Lederle "Laughlen reagent" at present warns against using this test on unheated blood samples and spinal fluid. Furthermore, all the above-mentioned investigators,[1-4] recommended the Laughlen test for use in the doctor's office by physicians, and even by those who had no special training in serology. These unfortunate statements, however, have been retracted, and in recent months there have been no further exaggerated claims for the Laughlen test.

Since 1938 a number of reports on the Laughlen reaction have appeared in the literature. Several investigators have found the Laughlen test to be satisfactory under laboratory conditions, while other workers have thoroughly condemned the method. Muether and Greutter,[5] after studying 1,000 blood specimens, concluded that the Laughlen test "is somewhat less accurate" than the Kahn test but "compares fairly well" with the Kline test. Dienst and Sanderson[6] examined the blood sera of 276 known syphilitic patients, who had positive clinical manifestations of the disease, and found that the Laughlen test had a sensitivity of 96.3 per cent compared to 93.4 per cent for the Kahn test, and 77.8 per cent for the Kolmer-Wassermann test. At the same time these authors tested 687 presumably negative sera and found only one false positive reaction. Breazeale, Greene, and Harding[7] performed the Kahn, Eagle, Ide, Kline, and Laughlen tests on 1,000 sera and showed an agreement between the tests of 98.4 to 99.5 per cent. Craig and Callaway[8] examined the unheated sera from 1,000 patients and found that the Laughlen test agreed with the Wassermann test in 91.8 per cent of the cases. With the Kahn test, however, a 99.8 per cent agreement was obtained. In 1939 a comprehensive study of the Laughlen test was published by Beck.[9] She found, in comparing the Laughlen test with 1,000 Kline tests, 1,100 Kolmer-Wassermann tests, and 100 Kahn tests, that the Laughlen reaction was just as specific (99.3 per cent) and somewhat more sensitive (77 per cent) than the other tests. Price[10] reported that the Kahn and Laughlen tests detect treated syphilitic persons better than the Wassermann test. He also found that the Laughlen and Kahn tests remain positive longer than the Wassermann test in cases of treated syphilis. Recently, another valuable survey of the Laughlen test was completed by Lever and Massie.[11] They performed Laughlen tests on the sera of more than 2,200 persons and compared their findings with the results of the Hinton and Wassermann tests. They found that in 442 presumably syphilitic patients the Laughlen test had a sensitivity of 75.6 per cent compared with 97.5 per cent for the Hinton test, and 42.8 per cent for the Wassermann test. In 1,778 nonsyphilitic patients these workers found only 0.7 per cent false positive Laughlen reactions. Lever and Massie

also reported that the Laughlen test is less sensitive but more specific than the rapid Hinton test, and less specific but more sensitive than the Kahn test. Taschner[12] compared the results of the Kline, Kahn, and Laughlen tests on 1,500 blood samples and found that the specificity of the Kahn and Kline tests was approximately 99.6 per cent, while that of the Laughlen test was 98.6 per cent. He, therefore, concluded that the Laughlen reaction is somewhat less specific than the Kahn and Kline tests.

On the other hand, a number of investigators have been unable to confirm the above-mentioned favorable findings. Usher[13] reported that the specificity of the Laughlen test is considerably less than that of the Eagle flocculation test. Furthermore, Usher found that when the sensitivity of the Laughlen reagent is reduced sufficiently to eliminate false positive reactions, it fails in a significant number of instances to detect sera giving a positive reaction with the Eagle test. Rein and Hazay[14] found the Laughlen reaction less specific and less sensitive than the Kline test. Flood and Mayer[15] examined 314 nonsyphilitic blood specimens and obtained four false positive reactions with the Laughlen test, whereas the Kahn, Kline, and Kolmer-Wassermann tests each gave three false positive results. On the basis of this small series, Flood and Mayer came to the statistically doubtful conclusion that the Laughlen test is less specific than the other three. These authors also found that the Laughlen test becomes negative earlier than the Kline, Kahn, and Kolmer-Wassermann tests during antisyphilitic treatment, and that the Laughlen test detects less early syphilis and less untreated syphilis than the other three tests. More recently, Moore[16] compared the Laughlen test with the Kolmer-Wassermann and Kahn tests and found the Laughlen reaction to be too erroneous to justify recommendation. Churg and Sobel[17] attempted to evaluate the Laughlen test by studying the reaction under conditions comparable to those in a physician's office or in a small laboratory. These authors compared their results with more than 2,000 routine Wassermann tests performed on the same specimens by the Serologic Laboratory of the City of New York. They found the Wassermann test to be more sensitive and more specific than the Laughlen test. In addition to these divergent results, editorial comments[18, 19] have appeared which warn against the use of the Laughlen reaction by untrained persons.

Thus, there seems to be no general agreement in the literature concerning the value and effectiveness of the Laughlen test. This is unfortunate, since a rapid, accurate, and easily performed test for syphilis would obviously find many uses at the present time. Such a test, if it were sufficiently sensitive and specific, would be valuable in emergency transfusions under both war-time and peace-time conditions, in certain preoperative situations, and for the legally required premarital serologic tests now common in many states. In the light of these considerations, and because of the conflicting reports in the literature, it was felt that it might prove useful to re-evaluate the Laughlen test by comparing it with the Hinton test in a large series of cases. The latter test, as it is performed in Hinton's laboratory, is undoubtedly one of the best flocculation tests for syphilis available at the present time.[20, 21] For the purpose of this study, 3,100 sera furnished by the Massachusetts State Laboratory were tested by Laughlen's method over a period of five months, and the results were compared with those

obtained in that laboratory by the Hinton reaction. Of these, 305 specimens were from proved syphilitic patients, almost all of whom had undergone various amounts and kinds of treatment. A total of 1,094 blood samples were from persons who had no clinical evidences or history of syphilis, and these are designated as "presumably negative." The remaining 1,701 sera were from persons whose medical history is unknown so far as syphilitic infection is concerned.

RESULTS

Of the 3,100 inactivated blood sera tested in this series, the results of the Laughlen test agreed with those of the Hinton test in 2,873 instances—an absolute agreement of 92.8 per cent. Of these, 609 were positive agreements, 2,256 were negative agreements, and 8 gave doubtful readings in both tests. Total disagreements numbered 227. These are analyzed in Table I.

TABLE I

227 CASES IN WHICH THE LAUGHLEN AND HINTON TESTS DISAGREED

Laughlen	+	0	+	?	0	?
Hinton	0	+	?	+	?	0
	64	70	21	13	22	37

Such data are not entirely satisfactory, since the actual sensitivity and specificity of the Laughlen test remain undetermined. There were 305 patients in this series, however, who had clinical manifestations of syphilis and whose blood sera showed a positive Hinton test. All but one of these had received some form of antisyphilitic treatment at some stage of the disease, and 198, or 65 per cent, were patients with neurosyphilis. In this group of 305 inactivated syphilitic specimens the Laughlen test gave 247 positive, 56 negative, and two doubtful readings, or a sensitivity of 81 per cent. This figure approximates the results obtained by Beck,[9] and Lever and Massie,[11] but falls far short of the sensitivity quoted by Dienst and Sanderson.[6]

To determine the specificity of the Laughlen reagent there were available 1,094 patients who had never had clinical manifestations or history of syphilis, and whose blood specimens yielded a negative Hinton test. In this series of presumably nonsyphilitic inactivated blood samples, the Laughlen test showed 1,077 negative, 12 positive, and five doubtful reactions, or a specificity of 98.4 per cent. This specificity is somewhat less than that quoted for the Laughlen test by other investigators,[6, 9, 11, 15] and no obvious explanation for this could be found. It should be mentioned, however, that two false positive results were from patients who are now known to have had active tuberculosis at the time the specimens were obtained. Parran and Emerson[22] have reported that false doubtful and false positive serologic tests are often encountered in persons with active tuberculosis.

Recently, Kirk and Bennett[23] described a rapid technique for testing small amounts of capillary blood with the Laughlen reagent. This method employs *noninactivated* blood samples, and although it has been generally conceded that the Laughlen reaction is thoroughly unreliable when used to test noninactivated sera, these authors found that the Laughlen test, performed by their method,

yielded entirely satisfactory results. In an effort to repeat the work of Kirk and Bennett, 577 blood samples were tested by the Laughlen reaction, using both heated and unheated sera. The results were then compared with those obtained with the Hinton test in the Massachusetts State Laboratory. In this series, when inactivated sera were used, the Laughlen test agreed with the Hinton test in 529, or 91.6 per cent, of the cases. Of these, 52 were positive agreements, 476 were negative agreements, and in one instance both tests gave doubtful results. This total absolute agreement does not differ significantly from that obtained in the entire series of 3,100 tests. There were 48 disagreements. These are set forth in Table II.

TABLE II

48 INACTIVATED BLOOD SERA WHICH YIELDED DIVERGENT LAUGHLEN AND HINTON TESTS

Laughlen	+	0	+	?	0	?
Hinton	0	+	?	+	?	0
	12	20	3	2	4	7

On the other hand, when the sera in this group were Laughlen tested without previous inactivation, the results agreed with the Hinton test in only 492 instances—an agreement of 85.2 per cent. There were 34 positive, 456 negative, and two doubtful agreements. The 85 disagreements are analyzed in Table III.

TABLE III

85 NONINACTIVATED BLOOD SERA WHICH YIELDED DIVERGENT LAUGHLEN AND HINTON TESTS

Laughlen	+	0	+	?	0	?
Hinton	0	+	?	+	?	0
	23	38	3	2	3	16

These results indicate that the absolute agreement between the Laughlen and Hinton tests is considerably less when the Laughlen reaction is performed with unheated blood sera. Heretofore it has been reported[9, 17] that it is the sensitivity of the Laughlen test which is decreased when noninactivated blood samples are employed. From Tables II and III, however, it seems that both the specificity and the sensitivity of the Laughlen reaction are seriously affected when unheated blood sera are used for the test. In addition, the difficulty of reading the Laughlen results is markedly increased when the test is carried out with noninactivated blood. This is suggested by the increased number of doubtful Laughlen readings in Table III.

In an attempt to determine further the effect of noninactivated blood sera on the specificity and sensitivity of the Laughlen test, blood samples were obtained from 31 known syphilitic patients and 350 presumably nonsyphilitic patients. These sera were then Laughlen tested before and after inactivation by heating at 56° C. for thirty minutes. The known syphilitic samples were from patients who had clinical manifestations of syphilis as well as positive Hinton tests. Presumably nonsyphilitic sera were from persons who showed no clinical evidences of syphilis and whose inactivated blood sera gave a negative Hinton test. In the small group of 31 known syphilitic blood samples the Laughlen test, performed on inactivated sera, showed 26 positive, five negative,

and no doubtful readings, or a sensitivity of 80.7 per cent. When the same syphilitic sera were Laughlen tested without previous inactivation, however, there were 19 positive, one doubtful, and 11 negative results, a sensitivity of only 61.2 per cent. In the series of 350 presumably nonsyphilitic blood specimens, the Laughlen test, after inactivation of the sera, yielded 343 negative, four positive, and three doubtful results for a specificity of 98 per cent. When the identical blood samples were tested by the Laughlen reaction, using unheated sera, there were 328 negative, nine positive, and thirteen doubtful readings for a specificity of 92.7 per cent.

The foregoing results reaffirm the conclusion that an attempt to develop a more rapid Laughlen technique by using unheated blood sera for testing is dangerous. Not only are the sensitivity and specificity of the test diminished, but the reaction is more difficult to read accurately when noninactivated sera are used.

COMMENT

The Laughlen test, possibly because of the exaggerated claims originally made for it, has not attained widespread clinical application up to the present time. The test is rapid and not particularly difficult to interpret, providing the observer has had sufficient experience. The antigen, as it is now supplied, is uniformly stable, and the preparation of the active antigen from the inactive material is relatively simple. Although the antigen must be activated at least twenty-four hours before use and although the active antigen is stable for only seven days, it is easy to prepare a week's supply of the reagent at one time. Such considerations would seem to make the Laughlen test particularly valuable for emergencies and for use in smaller outlying or isolated institutions, where it may be inconvenient or too expensive to perform other recognized tests for syphilis. Despite any claims to the contrary, however, considerable experience is necessary to read the Laughlen test accurately. In addition, the Laughlen test cannot safely be used to test spinal fluid or noninactivated blood serum.

From the confusing reports found in the literature, one cannot decide whether or not the accuracy of the Laughlen test justifies its acceptance. In general, it may be said that most of the evaluations which merely compare the percentage agreement of the Laughlen test with other well-known tests for syphilis on routine blood samples, are essentially unenlightening. A much more significant evaluation of any test for syphilis can be obtained by testing a series of known syphilitic and known nonsyphilitic blood samples, and expressing the results directly in terms of sensitivity and specificity.

In this study the Laughlen test performed on inactivated blood sera had an indicated sensitivity of 81 per cent, and a specificity of 98.4 per cent. This compares with a 77 per cent sensitivity and 99.3 per cent specificity obtained by Beck,[9] and a 75.6 per cent sensitivity and 99.3 per cent specificity reported by Lever and Massie.[11] From these results it seems that the Laughlen test is considerably less sensitive than the Kline and Hinton tests and approximately as sensitive as the Kahn test.[20] The specificity reported in this series is definitely less than that found by Beck, and Lever and Massie. Ordinarily, an acceptable

serologic test for syphilis must maintain a specificity of at least 99 per cent—a figure which the Laughlen test did not attain in our hands.

By its nature the chief attraction of the Laughlen test probably lies in its ability to exclude quickly the possibility of syphilis in certain clinical emergencies and diagnostic dilemmas. But the fact that the Laughlen test has an apparent sensitivity of only 75 to 80 per cent seriously detracts from its value in just those situations in which it should be most useful. On the other hand, if the Laughlen antigen is used in a supersensitive state, or if the results of the test are read closely enough to detect 95 to 100 per cent of syphilitic sera, then the specificity of the reaction falls markedly.* Nevertheless, the accuracy of the relatively simple Laughlen test is such that it may find a useful role in emergency situations, although its value in this respect probably does not equal that of the rapid Hinton test.[11] Greey, Bracken, and Paul[24] have recently indicated that the Laughlen test is a satisfactory method for detection of syphilis in blood donors, provided the serum is inactivated before testing. These authors also emphasize that the Laughlen reaction should always be confirmed by methods of greater specificity before a final diagnosis of syphilis is made.

If, as Dienst and Sanderson report,[6] the Laughlen test is 96.3 per cent sensitive and 99.9 per cent specific, then it probably is the best test for syphilis now available. No other investigators, however, have obtained results approximating these. On the other hand, to condemn the specificity of the Laughlen test as Flood and Mayer[15] have done on the basis that it showed four false positives whereas the Kahn, Kline, and Kolmer tests yielded only three false positives in 314 cases, is statistically unreasonable. The truth of the situation probably lies somewhere between these extreme viewpoints.

CONCLUSIONS

The Laughlen test is rapid and, in general, is approximately as easy to read as most acceptable flocculation tests for syphilis.

Of a total of 3,100 inactivated blood sera tested in this series, the results of the Laughlen reaction agreed with those of the Hinton test in 92.8 per cent of the cases. The sensitivity of the Laughlen test in 305 syphilitic patients was 81 per cent, and its specificity was 98.4 per cent in 1,094 nonsyphilitic persons.

The Laughlen test is thoroughly unreliable when used to test noninactivated blood sera. Not only is the sensitivity of the test markedly reduced, but its specificity is adversely affected, and the results of the test are more difficult to read.

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AUTOMATIC PIPETTE WASHER AND RINSER

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WE HAVE in this laboratory devised a pipette washer. This instrument, almost entirely automatic, requires less than three minutes of attention each day. By its use glassware can easily be made chemically clean. The instrument also saves some of the costs of cleaning fluid, water, and breakage. Since the full advantages of such an instrument may not be fully obvious, it may be of value to review, if only briefly, both the necessity for clean glassware and the methods by which chemical cleanliness may be obtained.

By definition glass is chemically clean when its surface is so free of extraneous matter that a film of water distributes itself over the surface of the glass in a layer, monomolecular in thickness. When a pipette is dirty, drop-

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lets, however small, remain attached to its surface. A dirty pipette will, therefore, not drain within its limits of error. In a micropipette the error may exceed 10 per cent. Since all chemistry, routine or research, is based upon quantitative analysis, it is absolutely necessary that all pipettes be chemically clean. So high a standard of cleanliness is not really difficult to achieve. All that is needed is a good cleaning fluid and an efficient rinsing system.

Chromic acid is the best cleaning fluid available. It is not, however, always made by the proper methods nor is it handled with the proper care. To make it cheaply and to handle it so that it may be efficient for as long as possible, the following technique should be used:

Five grams of technical potassium bichromate should be dissolved in a minimum amount of water. To this concentrated aqueous solution of potassium bichromate should be added one liter of technical sulfuric acid. Any amount may be made as long as the proportions given are followed. The final solution is dark brown, and in it may be seen the suspended red chromic anhydride crystals. The chromic anhydride is the oxidizing agent. When reduced, it turns to the green chromic oxide and is then useless for cleansing purposes and must, therefore, be discarded. The oxidizing capacity is destroyed by reducing agents, especially organic solvents as alcohol and acetone. All glassware should be rinsed before being placed in the chromic acid solution. The sulfuric acid is hygroscopic, and the solution will absorb atmospheric water unless the cylinder in which it is contained is closed to the atmosphere by a glass plate. Heat is produced when the acids are mixed. They may be mixed with safety if the cylinder is made of pyrex glass.

With the usual method of washing pipettes, each pipette is taken in turn from the acid solution. One end is connected to a suction apparatus, and the pipette is rinsed in running water. Since water is used for both the suction and the cleaning, and since each pipette is handled individually, the process is noisy, tedious, and wasteful. Some breakage is inevitable.

With the instrument described below, any number of pipettes can be cleaned, and yet no individual pipette is handled from the time it is soiled until it is again clean, dry, and ready for use. The system can run at night and, in all, requires less than three minutes of attention for any batch of pipettes. No breakage is possible during the cleansing process.

The complete apparatus consists of two glass cylinders, both made of pyrex and both of the same size; and a third smaller glass cylinder which sits upright in either of the other two. Since the smaller cylinder (marked B) is the most important part of the apparatus, it is described first.

During the working day cylinder B stands or lies on its side on the laboratory bench; as the pipettes are soiled, they are placed in it with their tips upward. The base of cylinder B is in the form of a hemisphere so that the mouthpiece of each pipette stands at a tangent. The passage of the water or cleansing fluid is completely free.

When it is necessary to clean the pipettes, cylinder B is lowered into cylinder A. Cylinder A is so constructed that there is an inlet of small caliber and a siphon outlet of large caliber. When the water is turned on, it fills both cylinders, its level rising until the bend of the top of the siphon is reached.

Both cylinders then empty, since the rate of outflow is faster than the inflow. That arm of the siphon which is inside cylinder A is grooved at its lower end so that when the water falls to this level, the air breaks the flow through the siphon. As soon as this occurs, both cylinders begin to fill again. Each filling and emptying requires between five and seven minutes so that it is possible to obtain ten or more washings per hour.

With the inlet of cylinder A attached to tap water, the pipettes are rinsed and drained, and the material so removed helps to preserve the cleaning fluid.

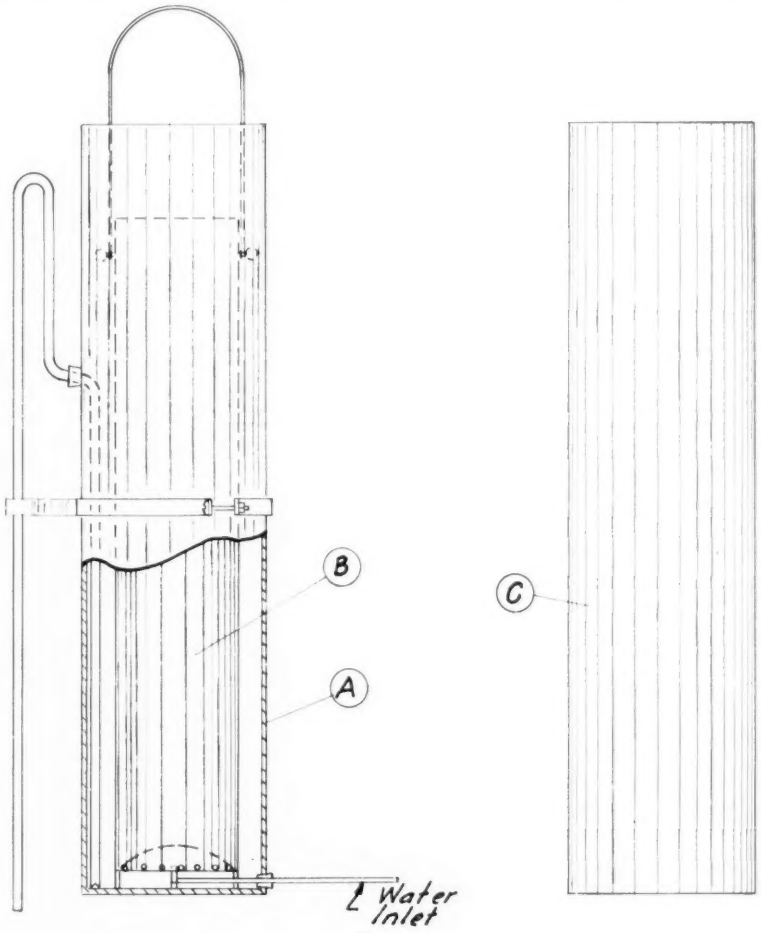

Fig. 1.

After three or more such flushings (the number is not of importance since the process is automatic) cylinder B is lifted free of cylinder A and allowed to drain, or else the siphon is permitted to empty both cylinders and the water is turned off. Cylinder B is then transferred to the chromic acid bath, cylinder C. The pipettes are usually permitted to stand in this acid for ten to twelve hours or overnight. When it is necessary to remove them, cylinder B is now raised by means of a lead handle, and the acid drains back into cylinder C. Cylinder B is now placed in cylinder A, the flow of tap water adjusted and the apparatus again permitted to fill and empty automatically. After the rinsing procedure

has continued for a sufficient time, the pipettes may be rinsed in distilled water by changing the connection at the inlet of cylinder A. A double connection, one for tap and the other for distilled water, may be used. Cylinder B may then be removed and placed in a drying oven. A glass plate may be used for covering cylinder B, which can then be permitted to remain on the laboratory bench. The pipettes will drain and dry, and, if time is not a factor, no gas oven is necessary. The height of the siphon can, of course, be adjusted so that short pipettes may be washed without the necessity of filling cylinder A to the top, saving, therefore, both tap and distilled water.

By the procedures outlined as used with the apparatus described, pipettes can be made chemically clean. The procedure requires only a simple technique which avoids breakage, saves time, and lowers laboratory expense, since it uses less tap water, distilled water, and cleansing fluid than any of the present methods. The pipettes rendered chemically clean by this method make quantitative analyses much more accurate.

75 Bay State Road

This apparatus is available through E. Machlett and Son, 220 East 23rd Street, New York, N. Y.

CHEMICAL

THE RELATIVE STABILITY AND POTENCY OF THROMBOPLASTINS FOR PROTHROMBIN TESTS*

HENRY G. PONCHER, M.D., JEANNETTE C. RICEWASSER, M.S., AND KATSUJI KATO, M.D., CHICAGO, ILL.

IN OUR preliminary report[1] we stated that thromboplastin solutions prepared from dried rabbit brain are remarkably stable. This finding is contrary to Quick's[2] statement, for we have observed that saline solutions of dried rabbit brain retain their potency for as long as six months. However, the duration of the thromboplastic potency of these solutions varies decidedly according to the method employed in preparing the original brain powder used in making the suspensions. In order to test the relative potencies of thromboplastins, and to ascertain the duration of their activities, the dried brain powders were prepared according to the two most commonly used procedures, namely: (1) the acetone-dehydration method of Quick, and (2) the incubator-desiccation method of Magath.[3]

EXPERIMENTAL

This investigation, conducted over a period of six months following the initial preparation of one composite of rabbit brains, consisted of 14 series of observations at biweekly intervals (Table I). The potency of each thromboplastic reagent prepared throughout this study was ascertained by determining its prothrombin clotting time with normal human plasma. The technique of prothrombin time used in this report is that of Quick's venous plasma dilution method.[4] Since the technical details are of paramount importance in an investigation of this nature, each step of the experimental procedures will be described somewhat at length.

Removal of the Brain.—From 8 to 10 c.c. of air were injected into the hearts of 18 normal rabbits by ventricular punctures, after which within one minute the animals usually died from air embolism. In an attempt to provide as bloodless a field as possible in the brain area, the animals were suspended by their necks immediately after death for approximately fifteen minutes. The brain was then removed from each animal in the following manner:

A longitudinal scalp incision was made between the occipital and frontal regions, so that the scalp could be separated into the lateral flaps. Using a rongeur, a niche was made in the parietal region of the skull. The brain was exposed by carefully chipping away the cranial bone, the movement being in a forward and lateral direction until the olfactory lobes were brought into view,

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and at the same time the meninges were also removed. The same procedure was carried out in a backward direction from the parietal region to the foramen magnum, thereby exposing the brain from the olfactory lobes to the spinal cord. Care was taken not to disturb the brain at any time while the rongeur was in the cranial cavity.

A small, flexible spatula was inserted below the olfactory lobes; by pushing it backward to the cerebellum the encephalon was freed from the base of the skull, except for the attachment at the optic chiasma. By moving the animal's head upward and backward through a 120° arc, the brain was easily severed from the optic chiasma. The animal's head was held in this backward position to allow the brain to fall into a position that would reveal the foramen magnum as well as the cranial nerves. By moving the spatula still farther back, the cranial nerves were cut, followed by a transection of the spinal cord just below the foramen. The final step was to cut the meninges at the tentorium cerebelli so that the brain could be completely removed from the cranium by a final upward movement of the spatula.

TABLE I

QUANTITATIVE DATA ON THE PREPARATION OF THE THROMBOPLASTIC SOLUTIONS AND PLASMA

SERIES	NO. PERIODS PER SERIES	EACH SOLUTION REQUIRED (C.C.)	EACH SOLUTION PREPARED (C.C.)	EACH BRAIN POWDER REQUIRED (GM.)	SALINE ADDED (C.C.)	TOTAL BRAIN POWDER REQUIRED (GM.)	PLASMA REQUIRED (C.C.)
I	14	5.6	11.2	0.56	10.64	2.24	1.6
II	13	5.2	10.4	0.52	9.88	2.08	4.0
III	12	4.8	9.6	0.48	9.12	1.92	5.6
IV	11	4.4	8.8	0.44	8.36	1.76	7.2
V	10	4.0	8.0	0.40	7.60	1.60	8.8
VI	9	3.6	7.2	0.36	6.84	1.44	10.4
VII	8	3.2	6.4	0.32	6.08	1.28	12.0
VIII	7	2.8	5.6	0.28	5.32	1.12	13.6
IX	6	2.4	4.8	0.24	4.56	0.96	15.2
X	5	2.0	4.0	0.20	3.80	0.80	16.8
XI	4	1.6	3.2	0.16	3.04	0.64	18.4
XII	3	1.2	3.0	0.15	2.85	0.60	20.0
XIII	2	0.8	3.0	0.15	2.85	0.60	21.6
XIV	1	0.4	3.0	0.15	2.85	0.60	23.2

Preparation of the Brain Powder.—After stripping the brains of the remaining fragments of the meninges and the discernible blood vessels, they were macerated to a smooth paste in a large mortar. This composite of fresh rabbit brains, weighing altogether 97 Gm., was equally divided into two portions. One portion was spread in a thin film over a glass plate, in accordance with the technique described by Magath,[3] and dried at 37.5° C. for sixteen hours. The dried brain paste was then removed from the glass by scraping it off in fine flakes with a small flexible spatula. The second portion was dehydrated with acetone and dried as recommended by Quick.[2]

The Magath and Quick thromboplastins were again divided into two equivalent portions; one part of each preparation was stored at 5° C. in separate tightly stoppered bottles and the other part was kept at room temperature in well-stoppered containers. From this original composite of fresh rabbit brain four types of thromboplastic material were thus available for investigation.

namely: (1) Quick's brain powder stored at 5° C.; (2) Quick's brain powder kept at room temperature; (3) Magath's brain powder stored at 5° C.; and (4) Magath's brain powder kept at room temperature.

Preparation of the Thromboplastic Reagents.—At fortnightly intervals adequate amounts of the four types of thromboplastic material for each series (Table II) were suspended in 19 parts of freshly prepared 0.85 per cent sodium chloride solution, thus making a 1:20 solution.* To insure a uniform preparation of all the thromboplastic reagents throughout this investigation, the suspensions of dried rabbit brain were always made in 9 by 65 mm. test tubes (Kolmer serum tubes). After thorough mixing, the saline suspensions of thromboplastin were warmed for fifteen minutes at 45° C. and then were again thoroughly mixed and finally allowed to stand for sedimentation of the coarse particles. The milky supernatant liquid from each of the four types of brain powder was then transferred to stoppered vials. For one-half of the experiments it was necessary to use several Kolmer serum tubes in order to make enough of one sample of a single thromboplastic reagent for a given series. After the sedimentation of the coarse particles the supernatant liquids from one type of rabbit brain powder were pooled together in the same vial.

Preparation of the Chemical Reagents.—The chemical reagents required in the performance of the prothrombin clotting time determinations in the present investigation were prepared according to the following specifications:

(1) M/40 calcium chloride solution: 0.368 Gm. of $CaCl_2.2H_2O$ (Baker's special grade) dissolved in 100 c.c. of distilled water.

(2) Physiologic saline: 0.85 Gm. of Merck's reagent NaCl in 100 c.c. of distilled water.

(3) 1.34 per cent sodium oxalate solution: 1.34 Gm. of $Na_2C_2O_4$ (Baker's analyzed grade) in 100 c.c. of distilled water.

Collection of Plasma.—For each series of experiments sufficient blood was drawn from patients with hypertension. If great difficulty was encountered in introducing the needle into the vein, the sample of blood was rejected. A freshly sterilized clean needle and syringe were used for each new venipuncture attempted at a different site. The blood was transferred without delay from the syringe into a tube containing 1.34 per cent sodium oxalate in such proportions as to furnish 1 part of anticoagulant to 9 parts of blood. After thorough mixing of the contents, the tube was centrifuged for fifteen minutes at 2,000 r.p.m. for complete separation of the plasma. Except for the 2 c.c. that was to be immediately used for the analysis, the stock supply of plasma was kept at 5° C. to prevent any possible diminution of its prothrombin activity, as well as any possible deterioration of the plasma itself. As more plasma was needed for the series of tests, 2 c.c. portions were removed from the stock supply.

Assay of the Thromboplastic Material.—The tests performed to assay the thromboplastic solutions thus prepared were carried out at 37.5° C. ± 1° C. One-fortieth molar calcium chloride solution was kept in a constant temperature water bath throughout the period of examination.

*Experience with various dilutions of rabbit brain powder with normal saline has shown a proportion of 1:20 (Kelley and Bray[5]) to give the same prothrombin clotting time as that of 1:18 dilution (Quick).

TABLE

PROTHROMBIN TIME IN SECONDS

(ONLY EVERY OTHER SERIES

DATE OF ASSAY PERIOD	SERIES I			SERIES II			SERIES IV			SERIES VI		
1940												
4/19	Q5	(F-F)	18.0									
	Qr	(F-F)	18.0									
	M5	(F-F)	27.2									
	Mr	(F-F)	27.7									
5/ 3	Q5	(14-14)	17.9	Q5	(14-F)	18.6	Qe		16.9			
	Qr	(14-14)	17.6	Qr	(14-F)	18.4						
	M5	(14-14)	26.2	M5	(14-F)	27.0	Me		24.7			
	Mr	(14-14)	25.4	Mr	(14-F)	27.1						
5/31	Q5	(42-42)	18.6	Q5	(42-28)	19.2	Q5	(42-F)	20.0	Qe		18.0
	Qr	(42-42)	17.4	Qr	(42-28)	17.8	Qr	(42-F)	22.5			
	M5	(42-42)	21.3	M5	(42-28)	27.7	M5	(42-F)	28.9	Me		25.7
	Mr	(42-42)	24.6	Mr	(42-28)	24.4	Mr	(42-F)	30.4			
6/28	Q5	(70-70)	17.5	Q5	(70-56)	18.3	Q5	(70-28)	19.7	Q5	(70-F)	19.8
	Qr	(70-70)	16.1	Qr	(70-56)	17.0	Qr	(70-28)	19.9	Qr	(70-F)	23.8
	M5	(70-70)	22.6	M5	(70-56)	28.1	M5	(70-28)	23.9	M5	(70-F)	31.8
	Mr	(70-70)	24.8	Mr	(70-56)	25.4	Mr	(70-28)	26.8	Mr	(70-F)	27.4
7/26	Q5	(98-98)	20.6	Q5	(98-84)	25.7	Q5	(98-56)	20.2	Q5	(98-28)	18.0
	Qr	(98-98)	20.4	Qr	(98-84)	20.9	Qr	(98-56)	27.3	Qr	(98-28)	22.9
	M5	(98-98)	38.0	M5	(98-84)	40.1	M5	(98-56)	36.9	M5	(98-28)	23.8
	Mr	(98-98)	31.0	Mr	(98-84)	>40.0	Mr	(98-56)	33.5	Mr	(98-28)	25.1
8/23	Q5	(126-126)	20.3	Q5	(126-112)	25.8	Q5	(126-84)	22.8	Q5	(126-56)	18.5
	Qr	(126-126)	21.3	Qr	(126-112)	20.1	Qr	(126-84)	>40.0	Qr	(126-56)	25.0
	M5	(126-126)	>40.0	M5	(126-112)	>40.0	M5	(126-84)	>40.0	M5	(126-56)	24.7
	Mr	(126-126)	>40.0	Mr	(126-112)	>40.0	Mr	(126-84)	>40.0	Mr	(126-56)	27.5
9/20	Q5	(154-154)	20.1	Q5	(154-140)	25.8	Q5	(154-112)	22.3	Q5	(154-84)	17.8
	Qr	(154-154)	20.2	Qr	(154-140)	19.9	Qr	(154-112)	>40.0	Qr	(154-84)	25.3
	M5	(154-154)	>40.0	M5	(154-140)	>40.0	M5	(154-112)	>40.0	M5	(154-84)	26.5
	Mr	(154-154)	>40.0	Mr	(154-140)	>40.0	Mr	(154-112)	>40.0	Mr	(154-84)	29.9
10/18	Q5	(182-182)	19.0	Q5	(182-168)	25.0	Q5	(182-140)	21.0	Q5	(182-112)	17.1
	Qr	(182-182)	19.3	Qr	(182-168)	19.5	Qr	(182-140)	>40.0	Qr	(182-112)	26.5
	M5	(182-182)	>40.0	M5	(182-168)	>40.0	M5	(182-140)	>40.0	M5	(182-112)	24.0
	Mr	(182-182)	>40.0	Mr	(182-168)	>40.0	Mr	(182-140)	>40.0	Mr	(182-112)	26.5

Abbreviations: Q and M indicate Quick's and Magath's thromboplastins. 5, r, and c, immediately following Q and M designate the brain powder kept at 5° C., at room temperature,

In a duplicate setup 0.1 c.c. of plasma was introduced into each serum tube (9 by 65 mm.) containing 0.1 c.c. of physiologic saline, followed by 0.1 c.c. of the thromboplastin to be tested.

After permitting the thromboplastin-plasma mixture to stand at room temperature for fifteen minutes, the tubes were warmed in the 37.5° C. water bath for one minute before the actual test was performed for each tube so prepared. The final step was the addition of 0.1 c.c. of calcium chloride solution, and at the same instant the stop watch was started. The tube was kept in the bath for the first five seconds* and was then held up against a source of light to insure good visibility of its contents. The tube was slowly inverted (about once in two

*Preliminary tests have shown that the prothrombin time values obtained by this technique were identical with those obtained by keeping the tube fixed in another large tube containing water at 37.5° C. and slowly inverted.

II

OF AGING THROMBOPLASTINS

AFTER SERIES II IS INCLUDED)

SERIES VIII			SERIES X			SERIES XII			SERIES XIV				
Qc		16.5											
Mc		24.1											
Q5	(98-F)	19.1	Qc		18.8								
Qr	(98-F)	25.1											
M5	(98-F)	23.8	Mc		21.0								
Mr	(98-F)	24.3											
Q5	(126-28)	19.1	Q5	(126-F)	21.7	Qc		17.2					
Qr	(126-28)	24.9	Qr	(126-F)	20.1								
M5	(126-28)	25.0	M5	(126-F)	23.2	Mc		22.2					
Mr	(126-28)	25.0	Mr	(126-F)	23.8								
Q5	(154-56)	19.6	Q5	(154-28)	27.2	Q5	(154-F)	20.6	Qc		18.2		
Qr	(154-56)	26.1	Qr	(154-28)	22.2	Qr	(154-F)	33.5					
M5	(154-56)	25.2	M5	(154-28)	25.4	M5	(154-F)	28.5	Mc		25.4		
Mr	(154-56)	27.7	Mr	(154-28)	28.5	Mr	(154-F)	26.8					
Q5	(182-84)	19.6	Q5	(182-56)	29.4	Q5	(182-28)	21.6	Q5	(182-F)	20.8	Qc	15.2
Qr	(182-84)	26.6	Qr	(182-56)	20.3	Qr	(182-28)	32.0	Qr	(182-F)	34.8		
M5	(182-84)	22.3	M5	(182-56)	24.6	M5	(182-28)	24.0	M5	(182-F)	26.0	Mc	25.1
Mr	(182-84)	24.2	Mr	(182-56)	27.7	Mr	(182-28)	27.2	Mr	(182-F)	28.7		

and the control. The figures enclosed in the parentheses represent the ages in number of days of the brain powders and of saline suspensions, respectively; F designates a fresh preparation. The sign > means greater than.

seconds) until the final reaction (clot formation) took place, the watch being simultaneously stopped. The results presented in Table II are averages of duplicate values which checked with each other within one second.

Plan of Experiment.—The prothrombin times obtained with fresh preparations of Magath's and Quick's thromboplastins with normal adult human plasma were noted first. The brain powders and the saline solutions were then stored in stoppered vials. All thromboplastin suspensions and part of each type of brain powder were kept at 5° C., the other part being stored at room temperature.

Two weeks later the activities of the original extracts, as well as those freshly prepared from another portion of the original composite of brain powder (by this time two weeks old), were tested on aliquots of one sample of normal plasma. Using this same sample of plasma, freshly made thromboplastin suspensions

from new Magath and Quick brain powders were tested for use as control values. This same plan of investigation was repeated at fortnightly intervals over a period of one hundred and eighty-two days (twenty-six weeks with thirteen biweekly testings).

For each experiment all the thromboplastic extracts on hand from the original composite of brain powder were tested and compared with a new solution of thromboplastin made from freshly prepared rabbit brain powder.

COMMENT

The experimental plan employed in the present investigation thus permitted the comparison of the stability of thromboplastic suspensions of various ages (multiples of fourteen days), prepared according to Quick's and Magath's techniques, at biweekly intervals and all from one progressively aging source of rabbit brain powder. This procedure also enabled us to ascertain the duration of the activities retained by Quick's and Magath's thromboplastin suspensions stored at 5° C., and to estimate the stability of suspensions freshly made from brain powders kept at this low temperature as compared with those stored at room temperature.

From the data presented in Table II, the two types of preparation thus tested exhibit decided variation in thromboplastic potency. It is apparent that the rabbit brain powder as prepared by Quick's technique of acetone dehydration is not only more potent in a fresh state than it is when prepared by the Magath incubator-desiccation method, but the saline suspensions made from the Quick powder retain their activity for a much longer period than those made from the Magath preparation. The slight decrease in thromboplastic activity of Quick's brain powder, as evidenced by the negligible prolongation of the prothrombin clotting time, is of no practical importance. It is, however, also evident that Magath's brain powder retains its thromboplastic potency in the same corresponding degree as Quick's preparation, since the prothrombin time obtained by a suspension made from aged powder is practically identical with that obtained by a similar solution prepared from fresh powder. In either instance, in order to safeguard retention of its potency, rabbit brain powder must be stored at icebox temperature in a well-stoppered vessel, since at room temperature changes detrimental to its activity invariably occur.

SUMMARY

Experimental data obtained in 14 series of observations made at biweekly intervals are presented, showing the relative potency and stability of thromboplastins made from both fresh and aging rabbit brain powders prepared according to the techniques of Quick and of Magath.

The results of our experiments demonstrate clearly the greater thromboplastic activity of rabbit brain powder prepared by the acetone-dehydration method of Quick than that of the incubator-dried preparation of Magath. Providing the preparation is stored at 5° C. in a tightly stoppered bottle, this superior potency of Quick's brain powder is retained for a period of at least six months. Likewise, if kept at 5° C., the suspension of rabbit brain powder, whether freshly made or as old as six months, is still capable of yielding pro-

thrombin clotting times practically identical with those obtained by it in its fresh state. The stability of both the dried rabbit brain powder, and its thromboplastic suspensions, which has been demonstrated in the present study, should dispel any doubt in regard to this particular feature and thereby encourage performance of prothrombin tests.

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A NEW STABILIZING AGENT FOR NESSLERIZED SOLUTIONS*

Its Application to the Determination of Urea Nitrogen in Biological Fluids

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IN THE visual comparison of nesslerized solutions, the eye of the observer is estimating extinction in a region of the spectrum to which the eye is least sensitive (absorption in the violet). For this reason, various substances such as gum arabic and gum ghatti can be added to stabilize the nesslerized solution without altering appreciably the precision of the visual comparison. The same substances, however, affect the photoelectric transmission value markedly. This is presumably the reason why some investigators in applying photoelectric techniques to the nesslerization of ammonia formed from blood urea have eliminated the gum stabilizing agent, but in doing this they have eliminated an important advantage enjoyed in the visual comparison, namely, stability of the transmission value of the nesslerized solution. Thus, Hoffman,[4] in eliminating gum ghatti, found it necessary to read his samples two minutes after color development and even then could read to only 40 mg. per 100 c.c. (0.016 mg. of urea nitrogen in 10 ml. of solution) of urea nitrogen in blood presumably because of rapid clouding of the sample. In addition, the transmission value of his blank is rather large, i.e., about 71 per cent. Nevertheless, the method is one of the most recent and successful in applying the photoelectric colorimeter to a direct nesslerization procedure for blood urea. In an effort to get back to the advantages of the visual comparison of nesslerized solutions and combine them with

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the advantages of the photoelectric colorimeter, we have sought a more efficient stabilizing agent. This paper sets forth what we believe to be our successful findings.

APPARATUS AND REAGENTS

Photoelectric Colorimeter. An instrument similar in design to the Evelyn colorimeter[1] was constructed with a sleeve of variable aperture to take a standard (16 by 150 mm.) pyrex tube rather than the large 25 ml. capacity tube used by Evelyn. However, any photoelectric colorimeter now on the market may be used instead of the instrument described.

Colorimeter Tubes. Tubes are selected from a large batch of pyrex (16 by 150 mm.) tubes and have graduation marks at 5 and 10 ml. They are chosen so as not to differ in transmission by more than 0.2 per cent.

Filter. The proper filter for the yellow nesslerized solution was selected from spectrophotometric data obtained with a Coleman-Bell double monochromater spectrophotometer. Using Corning glass filters, it can be composed by using 2 mm. of glass No. 585 with 3 mm. of glass No. 428. This combination gives a transmission band whose midpoint falls at about 440 mμ.

Water. Triple distilled, ammonia-free water was used for all reagents, standard solutions and dilutions.

Liquoid (Sodium polyanetholsulfonate). Hoffmann-La Roche product, 2 per cent solution in water.

Urease.[6] Treat 10 Gm. of permutit in a liter flask with 100 ml. of 2 per cent acetic acid. Decant off the acid, and wash the remaining permutit three times with distilled water. Add 150 ml. of 0.001 N sulfuric acid and 50 Gm. of jack bean meal and shake for fifteen minutes. Add 200 ml. of glycerol and shake in a shaking machine for one and a half hours. Allow mixture to settle for about one-half hour in the icebox. Then remove supernatant fluid and centrifuge it at 1,500 r.p.m. Transfer the supernatant fluid from the centrifuge tubes to glass stoppered botttles and place them in the icebox. This concentrated extract of urease will keep indefinitely.

Blood Filtrate Reagents. Tungstic Acid: This is a slightly more concentrated form of the Van Slyke and Hawkins[9] modification of the original Folin-Wu reagents and is made from two solutions: Solution A—Dissolve 111 Gm. of sodium tungstate ($Na_2WO_4.2H_2O$) in water and dilute to one liter. Solution B—Add 6 ml. of concentrated reagent grade sulfuric acid to 1,500 ml. of water and dilute the two liters.

For use, mix one volume of solution A with eight volumes of solution B. For blood filtrate, mix nine volumes of this mixture with one volume of blood.

Somogyi[7] reagents for zinc filtrate of blood: Solution A—10 per cent zinc sulfate: 100 Gm. $ZnSO_4.7H_2O$ dissolved in one liter of solution. Solution B—0.5N sodium hydroxide. Ten milliliters of solution A should require between 10.8 and 11.2 ml. of solution B to produce a permanent phenolphthalein end point. To prepare 1:10 blood filtrate, take one volume of blood with seven volumes of water. Add one volume of solution A, mix, and then add one volume of solution B. Mix again and filter.

Nessler's Reagent. Sixty-eight grams of the dry Nessler salt (obtained from the Technicon Co., New York City) is dissolved in one liter of 5 per cent sodium hydroxide. Allow to stand several days in a pyrex flask in a dark closet before using. Use the supernatant fluid without disturbing the precipitate.

Since the intensity of color development varies with alkalinity of reagent, an optimum alkali concentration was selected at 5 per cent with the help of the data presented graphically in Fig. 1.

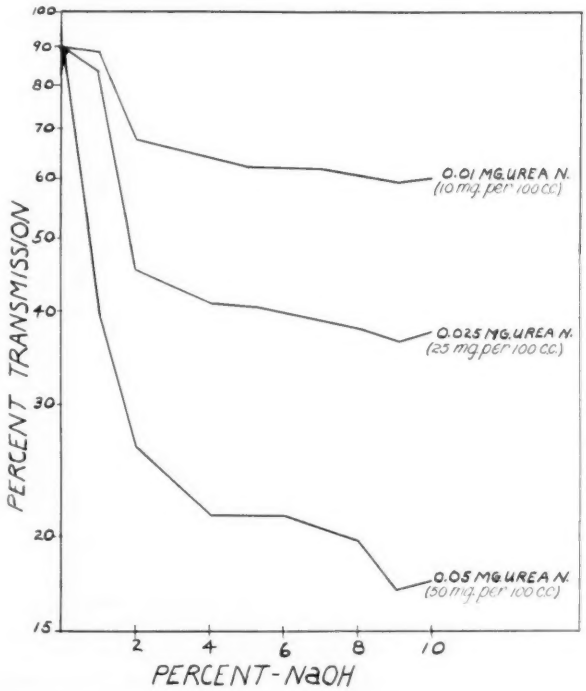

Fig. 1.—Alkali concentration of Nessler's reagent as per cent of sodium hydroxide solution (0.5 ml. Nessler's reagent in 10 ml. dilution).

Standards. Into volumetric flasks weigh out 3.821 Gm. and 2.145 Gm., respectively, of ammonium chloride and urea, dissolve in water, and dilute each to one liter. These were the stock standard solutions of which 1 ml. was equivalent to 1 mg. of nitrogen. (This value for the urea nitrogen stock standard was independently checked by a digestion, distillation, and titration procedure.) Appropriate quantities of 1:10 and 1:100 dilutions of these stock standards were pipetted out for the standards used in making the standard curves. The ammonium chloride and urea were recrystallized from reagent grade chemicals and dried at 110° C.

Stabilizing Agent. Since in nesslerized solutions clouding appears to be a process of gradual agglutination, the possibility of preventing it by means of blood anticoagulating agents was investigated. Liquoid (sodium polyanetholsulfonate) proved to be particularly effective. It gives very low blanks, is obtainable in pure form, and requires only one drop of a 2 per cent solution to stabilize a 10 ml. volume of nesslerized solution. An idea of liquoid's ability to prevent

cracking of a nesslerized sample can be gained from the fact that 0.25 mg. of ammonia nitrogen in 10 ml. of nesslerized solution could be stabilized for a maximum of sixty minutes with one drop of 2 per cent liquoid, whereas the same solution without liquoid cracked within thirty minutes. In addition, the transmission value stabilized with liquoid falls much more slowly than with the more frequently used stabilizing agents, such as gum ghatti, gum arabic, gum senegal,[2] etc.

PREPARATION OF STANDARD CURVES

Our particular field of application of nesslerization is the direct determination of urea nitrogen in blood filtrate. Therefore, in the preparation of the standard curves an effort was made to have the standard solution conform to the actual condition of a blood filtrate. To this end, the average concentration of the tungstic acid remaining in a blood filtrate was determined[3] and found to be about one-fourth that used in the precipitation of the blood proteins. This quantity, therefore, was added to each standard solution. In Somogyi filtrates the concentration of the remaining zinc ion is small and can be approximated by adding 1 ml. of filtrate obtained from the mixing of the Somogyi reagents with distilled water used in place of blood.

Standard Curves for Zinc Filtrates.—Into tubes graduated at 10 ml., quantities of standard urea solution were pipetted so that the nitrogen concentrations (after treatment with urease) would be those contained in 0.1 ml. samples of blood, ranging from 5 to 70 mg. per 100 c.c. of urea nitrogen. One drop of urease extract was added to each tube and after fifteen minutes' standing at room temperature, the tube contents were treated with the Somogyi filtrate reagents, mixed, and filtered through Whatman No. 2 (9 cm.) filter paper. One milliliter of this filtrate was placed in a colorimeter tube and one drop of liquoid was added. Five milliliters of water and 0.5 ml. of Nessler's solution were next added, and the tube contents were diluted to 10 ml. and mixed. Each standard was run in triplicate and read in the colorimeter at various time intervals against a distilled water blank.

To gain an idea of the thoroughness of conversion of the urea to ammonia by this technique, quantities of ammonium chloride standards were pipetted into a second series of colorimeter tubes so that the ammonia nitrogen would be that contained in 1 ml. samples of blood filtrate (equivalent to 0.1 ml. blood) resulting from bloods ranging from 5 to 70 mg. per cent in urea nitrogen. One milliter of zinc hydroxide filtrate obtained from the mixing of the Somogyi reagents (water replacing blood) was added to each tube followed by the addition of one drop of liquoid solution, 5 ml. of water and 0.5 ml. of Nessler's solution. The tube contents were diluted to 10 ml., mixed, and read in the photoelectric colorimeter at various time intervals. Each standard was run in triplicate. The transmission values of *both* these sets of standards fall on the same curve. The thirty-minute values are plotted in Fig. 2, where the coincidence of both sets of values is illustrated.

Another set of values was obtained for the same series of ammonium chloride standards used above, with 1 ml. of a 1:4 dilution of tungstic acid

filtrate reagent (water replacing blood) substituted for the zinc hydroxide filtrate used. The thirty-minute transmission values for these are also plotted in Fig. 2, and it is seen that they fall on a different curve. No urea nitrogen values are given on actual tungstic acid filtrates because the products of the enzymatic digestion of urea with the urease extract do not give a clear filtrate in the absence of blood or other protein-containing material. However, by analogy from the coincidence of the two standard curves based on standards containing zinc hydroxide filtrate and good recovery values of urea nitrogen using the tungstic acid curve, it is reasonable to assume that the curve based on ammonium chloride standards and tungstic acid will give transmission values very close to those that would be obtained from equivalent quantities of urea treated with urease.

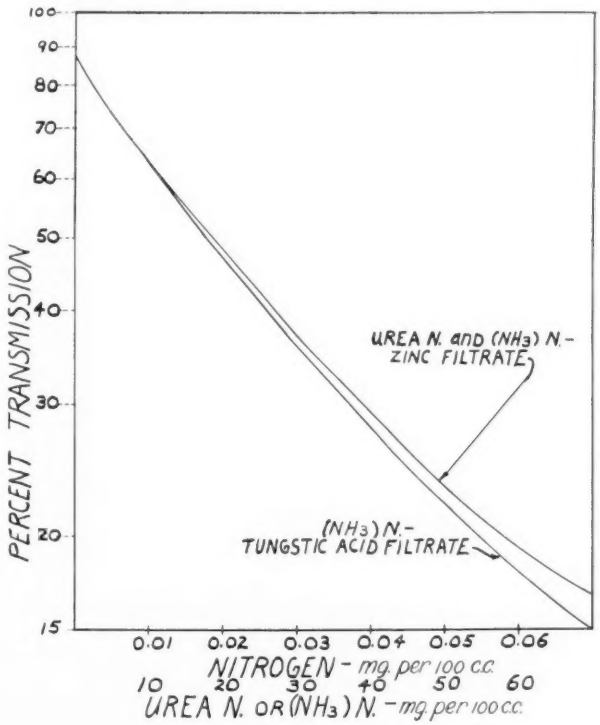

Fig. 2.—Transmission curves for colorimetric urea method for tungstic acid and zinc filtrates.

PROCEDURE FOR UNKNOWNS AND RECOVERIES

In the routine estimation of blood urea nitrogen 1 ml. of blood is treated in a test tube with 1 drop of urease extract and left to stand at room temperature for fifteen minutes. Nine milliliters of tungstic acid reagent (if zinc filtrate is preferred, zinc filtrate reagents may of course be used) are then added, and the mixture is well shaken. After another five minutes' standing, filter through a Whatman No. 2 (9 cm.) filter paper. One milliliter of the filtrate is placed in a colorimeter tube, followed by 1 drop of liquoid, 5 ml. of water, and 0.5 ml. of Nessler's reagent. The tube contents are then diluted to 10 ml.,

TABLE I*

DETERMINATION OF UREA NITROGEN IN BIOLOGICAL FLUIDS

(0.01 mg. urea nitrogen is equivalent to 10 mg. per 100 c.c. urea nitrogen)

SPECIMEN	UREA NITROGEN IN SAMPLE (MG. PER 100 C.C.)		UREA NITROGEN ADDED (MG. PER 100 C.C.)	TOTAL FOUND (MG. PER 100 C.C.)	RECOVERED (MG. PER 100 C.C.)	PER CENT RECOVERED
	DISTILLATION	NESSLERIZATION				
Serum 1		27.4	10	38.0	10.6	106
			25	51.1	23.7	95
Serum 2	7.4	7.6	5	12.7	5	100
			10	17.7	10	100
			25	32.7	25	100
Serum 3	7.4	7.8	5	12.5	4.7	94
			10	17.5	9.7	97
			25	32.4	24.6	98.4
Blood 1	6.3	6.8	5	11.7	8.9	98
			10	17	10.2	102
			25	33.8	27.0	108
Blood 2	12.5	12.5	10	24.1	11.6	116
			25	38	25.5	102
			50	61	48.5	97
Plasma	5.6	5.3	5	10	4.7	94
			10	15	9.7	97
			25	30.7	25.4	101.6
Cbs† 1		7.6	10	18.3	10.7	107
			25	33.8	26.2	104.8
Cbs 2	12.5	13.4	5	19	5.6	112
			10	24.2	10.8	108
			25	38.8	25.4	101.6
Cbs 3	7.2	8.1	5	13.9	5.8	116
			10	18.5	10.4	104
			25	33.2	25.1	100.4
Pleural fluid	8.7	8.8	5	13.6	4.8	96
			10	18.3	9.5	95
			25	34.7	25.9	103.6
Urine 1	196.7	205	100	306	101	101
Urine 2	256.8	262	50	313	51	102

*Values given in this table are the average of three or more determinations.
†Cerebrospinal fluid.

mixed, and read in the colorimeter at the end of thirty minutes, or at whatever time interval the standard curve is made. Other biological fluids are treated in the same way. However, one to ten dilutions are first made on urine specimens to bring their urea nitrogen content down somewhere within the range of the standard curve. The diluted sample is then treated exactly as for blood. Occasionally samples will be obtained with urea values so high that it will be desirable to repeat the determination on a smaller sample. If, however, a smaller sample is not available, an aliquot (α) of the 10 ml. volume in the colorimeter tube is taken and placed in another colorimeter tube, and 0.5 $(1 - \frac{\alpha}{10})$ ml. of Nessler's reagent is added. The tube contents are then diluted to 10 ml., mixed, and read in the colorimeter. If the extra Nessler's reagent is omitted, the trans-

mission value of the diluted sample will be too high. By this means the range of the method can be extended to ten times the range of the standard curve, i.e., 700 mg. per 100 c.c.

In Table I are given the urea nitrogen values obtained from tungstic acid filtrates of various kinds of biological fluids and urea nitrogen recoveries on these same fluids. The base values of the urea nitrogen content of these various samples are compared with those obtained by distillation and titration of the ammonia resulting from the urease treatment of the same filtrates. Values obtained by both methods are seen to be in good agreement. The recoveries, together with these compared values, are good enough to justify the use of the tungstic acid-ammonium chloride standard curve.

DISCUSSION

The extra buffer solution that is customarily employed in urea determinations to provide the optimum pH for action of the enzyme, urease, is omitted, because with the concentrated stable extract of urease used, it adds nothing to the completeness of conversion of the urea present.

Tungstic acid filtrates instead of zinc filtrates are prepared in this laboratory where daily filtrates sometimes number in excess of 100, because they require only a single reagent, whereas zinc filtrates require at least two, and with the original Somogyi technique, three, if the distilled water for the proper volume dilution is considered. In using tungstic acid filtrate we did not encounter the high blanks of which Hoffman[4] complained.

Since the transmission value of a nesslerized solution falls slowly, the standard curve should be made for a definite time interval, and the unknowns should be read at this interval.

In the practice of photoelectric colorimetry some writers[8] criticize the use of standard curves because they feel that the conditions under which the curve is prepared at one time, and those under which an unknown is read at another, are never exactly duplicated, and, therefore, choose to compare their unknown with a freshly prepared standard, and to get their result from the familiar inverse proportion based on the Bouger-Lambert-Beer law. This is sound practice when the law holds. In defense of our using a standard curve, we wish to point out that in many cases the Bouger-Lambert-Beer law holds only in a limited range of lower concentrations and deviates considerably at higher concentrations. Therefore, calculations based upon it often contain more error than would be introduced by use of a standard curve whose duplicability had been checked over a long period of time. The standard curves illustrated in this paper have been used and checked over a period of two years and furnish, we believe, an example of a set of conditions that can be sufficiently duplicated to validate their use.

Although the standard tungstic acid curve presented is especially prepared for blood filtrates, and an amount of tungstic acid is placed in the standards that approximates that in blood filtrates, the curve is usable for estimating the urea in tungstic acid filtrates on biological materials other than blood. This is apparent from the results given in Table I, which were obtained by use of this curve.

SUMMARY

A new stabilizing agent, liquoid, is offered for nesslerized solutions that gives very low blanks and stable transmission values. The technique is applied to urea determinations on the filtrates of biological fluids that have been treated with urease.

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MEDICAL ILLUSTRATION

FLUOROGRAPHY*

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DEFINITION

FLUOROGRAPHY is the process of recording, by use of an optical system and light-sensitive film, the image formed upon a fluorescent screen (1) by a differentially absorbed beam of radiant energy, usually roentgen rays; or (2) by a stream of directed electrically charged particles. The process, then, includes the photographic recording of fluoroscopic images found in medical or industrial radiology, the photography of television images, the photography of cathode-ray oscillograph patterns, and similar images. Fluorography definitely does not encompass all types of photographic recording in which fluorescence is utilized. For example, contact radiography in which a light-sensitive film is most frequently, but not necessarily, sandwiched between two fluorescent screens is not considered to be fluorography; nor are such methods as described by Dershem,[1] wherein a directed beam of fluorescent x-radiation is used to record the distribution of certain elements in a thinly sectioned specimen, by selecting as the fluorescing substance an element whose characteristic K-radiation corresponds closely to the K absorption limit of the element to be detected; nor, in spite of the similarity of name, is the so-called fluoroscopic process,[2] invented by W. S. Marx, Jr., in 1938 in any way related to fluorography. This latter process is a special procedure for producing so-called "high-light" half-tone screen negatives for use in photomechanical reproduction.

The process now called fluorography is, however, known also as indirect radiography, fluoroscopic screen photography, fluorophotography, miniature radiography, fluororadiography, microradiography, and roentgen photography, and at one time was called photofluoroscopy. It is interesting to note that the term which has been adopted through usage is the most simple. At a time when there was some question as to the etymologic correctness of the term fluorography, I approached the heads of the English departments in three leading United States universities with the question independently, and in each case, after learning the nature of the process and reviewing the names which had at one time or another been used to describe it, these authorities selected fluorography as the most logical term to adopt.

HISTORY

Roentgen discovered x-rays in 1895. In the following year several means were devised for photographing the fluoroscopic image excited by these rays.

*From the Agfa Ansco Product Information Department, Binghamton.
Read at the Meeting of the Biological Photographic Association, September, 1941.

MacKay[3] described one method in March, 1896; Bleyer[4] constructed his photofluoroscope in April, 1896; Battelli and Garbasso[5] also described a method in January of the same year. In 1897 Porcher[6] published an article entitled, *Photographie de l'image fluoroscopique*. In 1897 McIntyre[7] published an article describing indirect radiographic motion pictures which he accomplished during a series of experiments in 1896 by photographing the shadow image formed on a potassium platino-cyanide fluorescent screen. At this period there was a sharp turning point with respect to the manner of making radiographic records. Initially, the photography of the shadow image formed on the fluorescent screen was the preferred method, but Porcher[6] and others were of the opinion that the results obtained in indirect radiography were unsatisfactory, and hence the direct method gained favor. A few years later Köhler[8] anticipated the promising future of fluorescent image photography; in 1909 Biesalski and Köhler[9] outlined a practical method for conducting the work in which were utilized a calcium tungstate screen (blue fluorescence) an F/2 lens, and a mirror permitting the camera to be placed outside the direct beam of roentgen rays. This revival of the indirect procedure by Köhler gained momentum as time went on, and within a few years nearly all the problems involved in the process had been recognized.

Caldwell[10] in 1911 published an important paper on indirect radiographic still photography and cinematography in which he set down practically all the essential principles concerning the application of fluorography. He employed a 4 by 5 inch camera and a F/4.5 Cooke lens in conjunction with a "Gehler Folie" for still work. The screen that Caldwell used had a short phosphorescent afterglow which, as pointed out by Hirsch,[11] anticipated the most suitable screen available today for fluorography—the "fluorazure" screen, more properly known as the photo-roentgen screen, which has an afterglow also, but as used in practice it does not persist long enough to interfere with the clarity of the recorded image.

Loman and Camandon[12] were the first to utilize an extremely wide aperture lens in fluorography. In 1913 they employed a lens of F/1.55 relative aperture for motion pictures of the fluoroscopic image.

It is doubtful if much of this early photography of the fluoroscopic image was successful, especially the motion picture photography.* The problems involved were well understood, but the limitations were too severe to permit satisfactory results. It was not until lenses of wide aperture were generally available, until photographic emulsions were made faster, and until more efficient roentgen-ray tubes and fluorescent screens were devised that fluorography produced results worthy of evaluation.

APPLICATIONS

Although categorically fluorography includes the photographing of fluorescent screen images of any type, the widest field of application is found in medical radiology. Nearly the entire development of the process has been con-

*This view is based somewhat upon the private communication from S. L. Warren, Rochester, N. Y., reported in the *Science of Radiology*, page 204, edited by Otto Glasser (1933), in which it is claimed that after five years' labor it had been found impossible to achieve a satisfactorily sharp image in cinefluorography with the use of lenses of very wide aperture. This statement had reference to the publication of Luboschez,[25] who had reported good results in 1929 by means of a lens of F/0.65 relative aperture.

tributed by medical researchers and roentgen-ray technicians, their reports being published in medical and allied journals. In medical radiology there are two distinct applications of fluorography, single and serial exposures. Both applications are important, and the same technical problems in attaining suitable results are involved. In serial fluorography the successive exposures frequently can be made sufficiently rapid to permit the taking and subsequent projecting of roentgen-ray motion pictures.* This branch is called cinefluorography.

Single exposures of the fluorescent image are made for purposes of record or diagnosis, or both. For group studies of large populations, as in thoracic surveys, fluorography represents a distinct advantage over contact radiography, especially from an economy standpoint. This branch is known as collective fluorography and the individual records are called fluorograms.

In Germany the application of fluorography in medical diagnosis is regarded as limited to the determination of lung shadows, disease centers of sizeable area, and the frequent detection of active tuberculosis. It is not considered suitable for showing satisfactory bone structure, nor early infiltrations in pulmonary disease, such as early tuberculosis and early silicosis.[13] However, Hirsch[11] in this country, with reservation similar to that of the Germans but with a somewhat more optimistic outlook, states that fluorography is: 1. Applicable to the study of the skeleton in delineating *gross* bone changes and deformities. 2. Useful in gastrointestinal surveys, particularly in the study of the colon and in motility tests. 3. Suitable for showing clearly the position and shape of the heart and, upon applying proper correction factors, the heart shadow diameters can be determined according to the usual method. 4. Valuable in the study of the lungs, particularly for tuberculous changes, the records being sufficiently clear to permit the differentiation of the normal from the abnormal lung.

Manoel de Abreu,[14] who in 1936 inaugurated the first installation of fluorographic apparatus for the purpose of carrying out a mass examination in Rio de Janeiro, also is of the opinion that fluorograms yield useful diagnostic evidence. His enthusiasm for the process is largely responsible for the installation of numerous fluorographic outfits throughout Brazil and other South American countries. Abreu's influence was not limited to South America, for he acquainted D. O. N. Lindberg of Decatur, Ill., with the success of his work, and Lindberg brought the method to the United States in 1937.

Cinefluorography has been found by Stewart and Ghiselin[15] to be safer and more effective than other radiographic procedures in the study of abnormal diaphragm and mediastinal movements in the understanding of pulmonary disease. In an earlier paper these same workers[16] reported that cinefluorography had several advantages over direct fluoroscopic examination in the study of many moving organs, such as the heart. They claimed, in addition to other factors, that the motion picture was even more defined than was observation with the best possible fluoroscopic screen. In a similar vein Böhme[16a] expressed the merits of cinefluorography in studies of the heart. He used the process to

*So-called x-ray or roentgen-ray motion pictures are not entirely "real." The motion picture record is actually only a repetition of a single cycle which gives the effect of a continuous action. However, single cycles of an organ do not constitute a full study of the organ, since some cycles may be normal whereas others may be pathologic.

demonstrate his discovery regarding the important function of the auriculoventricular diaphragm in the propulsion of blood. He was able to make as high as 150 exposures per second for short intervals of time, three to four seconds.

Outside the medical fields fluorography has had a scattered but significant application. With the coming of an increased use of fluoroscopic roentgen-ray examinations in industry for quality control, it is natural to assume that there would be a corresponding increase in the number of photographic records desired. Moreover, the growth in the uses for cathode-ray tubes has occasioned an increase of interest in retaining records of traces in certain experimental and even in routine studies.[17] Dudley[18] has published an article on the photographic recording of television images. Undoubtedly interest in this branch of fluorography will gain in application as television becomes more practical.

There are many optical and photographic problems which pertain to fluorography, and in view of the likelihood that the process will find wider application in medicine, industry, and science, it is deserving of more extensive study.

FACTORS IN OBTAINING OPTIMUM RESULTS

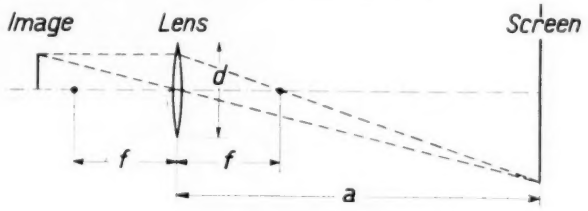
The aim in fluorography is specifically to reproduce as accurately as possible the fluorescent screen image with a maximum of sharpness and with adequate contrast to permit evaluation of details. In medical applications an additional aim, of course, is to replace radioscopic examination by a less dangerous method and, in addition, to offer better documentation.[19-21] Whether or not the aims are satisfied is determined by the conditions of operation and the acumen with which the situation as presented by the limitations of equipment is met. First results are often disappointing, simply from a lack of appreciation for the problems involved. The alacrity with which an individual technique is evolved will be influenced vastly by the extent of one's knowledge of the overall problem.

There are several factors that govern the quality of the result.[13, 19, 22-24]

1. The efficacy of the radiant energy or electron stream to excite fluorescence. In the case of medical radiology, the energy of the incident roentgen-ray beam is limited by the capacity of the tube and by the tolerance of the patient's skin.
2. The light-producing power of the fluorescent screen, which varies qualitatively and quantitatively according to:
   (a) The chemical composition of the screen.
   (b) The color energy distribution of the fluorescent radiation.
3. Sharpness factors which are dependent upon additional screen characteristics, namely,
   (a) Graininess of screen structure, whether coarse or fine.
   (b) Time lag of screen.
4. Characteristics of the camera lens which include:
   (a) Light-gathering power; that is, the lens speed.
   (b) Sharpness of the lens at wide apertures.
5. Sensitivity of the film to light energy emitted by the screen.

It is not convenient to discuss these various factors in the order given. Because the process of fluorography involves the use of an optical system, it is best

to outline first the limitations which this imposes. Moreover, it is the use of an optical system that chiefly distinguishes fluorography from normal roentgenographic procedures in which the recording film and screens are in actual contact. However, it is the matching of the quality of the large roentgenogram, made in the normal manner, by the indirectly made reduced image that more or less establishes the quality criterion for fluorography.



d equals aperture of lens
f equals focal distance
a equals screen-lens distance

$$\frac{\pi d^2}{4} : 2\pi a^2 \quad \text{or} \quad \frac{d^2}{8 a^2}$$

Fig. 1.

In contact radiography* the entire radiation within an angle of 180 degrees, and even more when screens are used on both sides of the film, is utilized in exposing the film. With a lens system the amount of radiation received by the film is limited at the outset by the acceptance angle of the objective. This requires, of course, a brighter screen image in fluorography than is necessary in normal roentgenography, but since this in turn demands an increase in the total energy output of the roentgen-ray tube, it is desirable to employ the fastest possible photographic objectives. Lenses of relative aperture in the range of from F/0.53 to F/0.625 have been used in cinefluorography,[25-27] but such lenses are not readily available nor could they be made of sufficient focal length to cover a large field. The Zeiss R-Biotar objective is available with a maximum F value of 0.85, 4.5, or 5.5 cm. focal length, but the overall sharpness cannot be regarded as entirely satisfactory. Much more satisfactory results are obtainable with the Zeiss Biotar F/1.4, 7 cm. focal length, or with the standard Contax, Leica, or Ektar F/1.5 to F/2.0 objectives. Furthermore, it is preferable to have even these objectives surface-treated to minimize internal light reflections, otherwise the resultant loss in image contrast would have to be gained through an increase in the brilliance of the screen image or by the use of a film of exceptionally steep exposure-density gradient.

A mathematical approach to the determination of the conditions for optimum results has been made by Bouwers,[19] which is an expansion of a former work concerning the so-called law of uniformity.[28] This law is concerned with the factors which are causes for unsharpness in the normal roentgenogram of objects in motion and specifically states that optimum conditions are reached when the factors are equal; thus,

$$U_m = U_g = U_s \qquad (1)$$

*It should be noted that television and other cathode-ray tube tracings can be recorded by contact methods but less conveniently than by means of a camera.

where U_m is the unsharpness resulting from movement, U_g is the geometrical unsharpness, and U_s the unsharpness caused by screen factors. Mean values of U_g and U_m are assumed since these factors are not constant for the whole image. The law of uniformity is based upon the fact that the product of U_m, U_s, and U_g is a measure of the amount of light reaching the film and, for a given value of the product, a given image density is achieved. However, the sum of three numbers with constant product is smallest when the numbers are equal; therefore, we get equation 1, since the total unsharpness is the *sum* of the three unsharpness factors.

Although the law of uniformity originally applied to normal radiography, it is equally applicable to fluorography, provided, as in the case of normal radiography, that a rotating anode tube is used. For example, if we assume that proper radiographic conditions for optimum results are established, we can eliminate one contact fluorescent screen and photograph the image on the remaining screen with a lens and camera. With the fastest practical lenses the light per square centimeter incident upon the film in the camera is only one-tenth that per square centimeter incident upon the film when in contact with two fluorescent screens. Accordingly, if a film of the same sensitivity as the normal roentgen-ray film were used, and the energy output from the tube remained constant, then an exposure increase of twenty times would be required for the camera exposure. However, such a vast increase in *time* of exposure would increase U_m to an intolerable extent and at the same time the life of the roentgen-ray tube would become endangered, not to mention the danger involved by giving the patient such a large roentgen-ray dose.

Fortunately, the law of uniformity can be applied to show what changes are required to establish exposure conditions for optimum results. Since

$$U_m = U_g = U_s$$

and since in the case at hand

$$U_m \times U_g \times U_s = 20X$$

the new conditions will be best if each unsharpness factor is multiplied by

$$\sqrt[3]{20} = 2.7$$

in order to increase its product by a factor of twenty. These conditions could be met by the use of a tube of wider focus, by means of a somewhat coarser grained screen of greater layer thickness, and by increasing the time of exposure now by only 2.7 times. The unsharpness in the final reduced image is then only 2.7 times greater than in the direct double screen image. From this it is apparent that the loss of sharpness in the small image is determined by the ratio of the amount of light per square centimeter in the small image, I′, to the amount of light, I, that would be available per square centimeter of film surface in the direct method. Therefore, if we denote the increase of unsharpness by the factor k, its magnitude is determined by

$$k = \sqrt[3]{\frac{I}{I'}} \qquad (2)$$

As previously mentioned, this deduction is based upon equal film sensitivities which at present, at least, can be assumed to be approximately true. There are

also additional factors that are ignored, otherwise the treatment becomes unduly complicated. For example, no regard is given the fact that fluorograms are projected and accordingly can be of a lower density level than normal roentgenographs.

The calculation of the ratio of I′ : I can now be considered. In Fig. 1 the geometric relation between the screen, camera lens, and reduced image plane are shown. If d is the aperture of the lens, f its focal distance, and a the screen lens distance, then the relation of the light passing through the lens to the total light emitted by the screen is

$$\frac{\pi d^2}{4} : 2\pi a^2 = \frac{d^2}{8a^2}$$

If the reduction is n times, then the surface area over which the light passing through the lens is distributed will be $\frac{1}{n^2}$ that of the screen; consequently, the brightness of the reduced image becomes $\frac{n^2d^2}{8a^2}$ times that in a film in contact with one screen, or $\frac{n^2d^2}{16a^2}$ times that in a film between two screens. But,

$$n \cong \frac{a}{f}, \text{ so } \frac{I}{I'} = 16\left(\frac{f}{d}\right)^2 \qquad (3)$$

Now, by equation 2 we obtain for the increase in unsharpness the factor,

$$k = \sqrt[3]{16\left(\frac{f}{d}\right)^2} \qquad (4)$$

It so happens that it is an advantage to use a higher voltage for fluorography than is favorable for direct roentgenography. By so doing an increase in sharpness follows, since the tube output is increased and results in a smaller product for $U_s \times U_m \times U_g$ for a given image density. This increase in tube voltage also decreases the factor I/I′. Now if in addition to this, the focus screen distance is reduced, a gain in light intensity can be effected. As a matter of fact, the sharpness can be doubled in the small image by increasing the tube voltage by 30 per cent and by reducing the focus-screen relation to one-half.

A new unsharpness factor can be established if these compromises are made, since equation 4 becomes

$$k' = \sqrt[3]{4\left(\frac{f}{d}\right)^2} \qquad (4a)$$

when the tube voltage is increased 30 per cent and the focus-screen distance is halved.

The values of $\frac{f}{d}$, as we have noted, are generally not smaller than about 1.5. Substituting this in formula 4a, the unsharpness factor becomes 2.08. This amount of unsharpness, according to Bouwers, corresponds to the relative sharpness acquired with rotating and stationary anode tubes.

From these calculations, which comply rather closely with practice, it is clear that there is room for improvement in the comparative quality of fluoro-

grams as made in medical roentgenography. However, the present results under the best conditions compare favorably with the quality of direct roentgenograms of an earlier period, and so for many applications can be considered adequate.

Sharpness Factors Dependent Upon Screen Characteristics.—In the foregoing calculations Bouwers[28] eliminated the unsharpness resulting possibly from the lens and film by assuming that these would be small in comparison to the unsharpness caused by other factors, even under conditions conducive to ideal reproduction. (The elimination of the lens as a factor becomes more acute in cinefluorography, for here the demand for wider aperture and greater sharpness are both encountered.) He assumed that miniature camera films of the fine grain type would be employed, but actually in modern fluorographic practice special films are coming to the fore. The films have an exceptionally high sensitivity to the fluorescence emitted by available photo-roentgen screens, and in addition are of rather steep inherent contrast. However, the graininess of these films compares favorably with the graininess of modern high speed roentgen films. Nevertheless, in relation to the unsharpness introduced by the grain structure of the screen as well as other factors, the film is well within the permissible limits as far as these concern the ability of the film to register fine detail, viz., resolving power. For example, if we assume the unsharpness in a normal radiographic image to be of the order of 1 mm.,* the magnitude of the unsharpness in a 15 diameter reduction would be about 0.07 mm. The resolving power of the film, then, is required to be less than 10 lines per millimeter, which is far inferior to present emulsion standards even for coarse grain films. This means that the extent factor of unsharpness here rests with the nature of radiographic imagery and the fluorescent screen. The size of the focal spot and other factors determine the sharpness of the image primarily, but the coarser the grain of the screen the less fine detail will be discernible. However, since increased light emission accompanies an increase in grain size of the screen, there must be an optimum condition that can be met. Actually, the emission characteristics of the screen should be a maximum for a given amount of excitation, but this should not be carried to a point that will give an unsharpness greater than that presently accepted in normal radiography. It would be ideal, of course, to have a screen of high emissivity and of very fine grain structure.

The phosphorescent lag of the screens used in modern roentgen-ray practice is generally nil. And certainly there can be no appreciable lag in the screens adapted for cathode-ray tubes in television and in oscillograph manufacture. However, as previously pointed out, with the special blue fluorescing Patterson photo-roentgen screen there is a persistence of image, but this is of no consequence in the methods used in still fluorography. If anything, the afterglow slightly increases the image density without causing deleterious effects. Screens that are employed in cinefluorography can exhibit no persistence of image. For this work the Patterson fluoroscopic, type B, screen is excellent. A special study of the lag effect has been undertaken by Hirsch.[29, 30] He found that by permitting the camera shutter to remain open five minutes after the short ex-

*It should not be inferred from this discussion that the screen is the chief cause of unsharpness in the normal radiographic image. The screen unsharpness is of the order of 0.15 to 0.17 mm., according to measurements made by the Patterson Screen Co. These data are corroborated by measurements published by H. Mermagen, x-ray technician, September, 1939.

citation period and by using a special screen with pronounced lag, an appreciable gain in image density resulted, but only at a sacrifice of overall contrast.

The Light-Producing Power of the Fluorescent Screen.—The exact chemical composition of commercial fluorescent screens is not available, nor is such information of particular value. It is known that certain compounds, when properly crystallized and dispersed, exhibit fluorescent properties to a greater or lesser extent, and that the color of the light varies according to the particular substance used. Roentgen-ray intensifying screens are dependent upon calcium tungstate for their fluorescence. Today these screens exhibit no lag, although at one time this represented a considerable problem. Minute quantities of certain metals are known to eliminate afterglow; for example, as small an amount as 10^{-6} Gm. of nickel added to a zinc sulfide layer is sufficient. However, regular intensifying screens are not used in fluorography. Instead, zinc sulfide fluoroscopic screens of either blue-green or blue fluorescence are preferred. The color energy distribution of the fluorescence for the various types of screens is given in Table I.

TABLE I

TYPE	SPECTRAL EMISSION
Intensifying screens (Calcium tungstate)	3,800 to 5,200 A. (Maximum band at approximately 4,300 A.)
Fluoroscopic screens (Patterson photo-roentgen or fluorazure)	3,900-4,900 A.
Fluoroscopic screens (Type B)	5,200-6,000 A.
	(No sharply defined maximum band is exhibited by either type of fluoroscopic screen.)

Sensitivity of Film to Light Energy Emitted by Screen.—Either standard film products or special films can be used in fluorography. It is preferable to use a film of high contrast, and one which is especially sensitive to the radiation emitted by the fluorescent screen. A film of this type, developed by Agfa Ansco, is known as "Fluorapid."

The spectral sensitivity of Fluorapid film is represented in the wedge spectrogram, Fig. 2. It is readily apparent that the major part of the sensitivity of the film is between 4,000 and 5,700 A. This range is suitable for both the photo-roentgen and type B fluorescent screens.

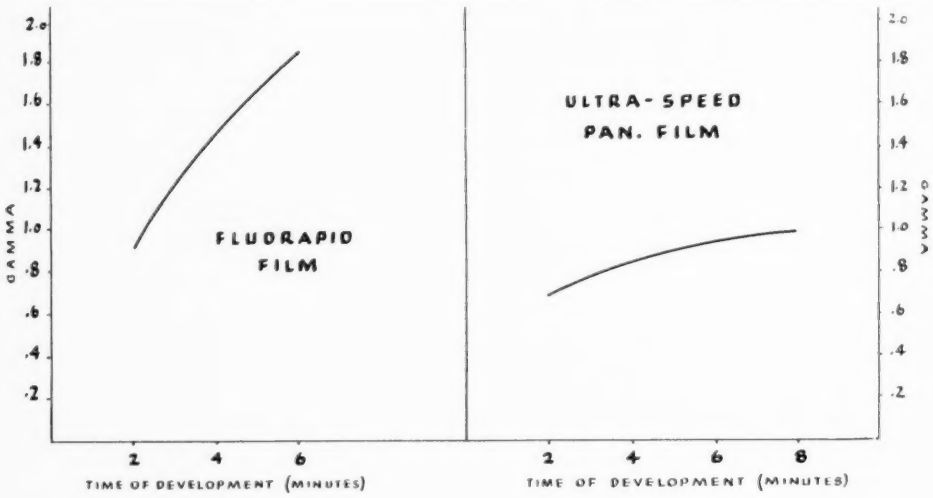
The total sensitivity of Fluorapid film to the light of the photo-roentgen screen is especially high, being equal to, or slightly faster, in the threshold region than in the high-speed roentgen-ray film emulsions. In addition to having a speed equal to the fastest emulsions available today, it also has a relatively steep exposure-density gradient. As shown in Table II, the film can be developed in roentgen-ray type developers to gamma values of over 1.8 which, in comparison to a regular miniature camera type film of similar speed, is exceptionally good.

Other types of films can be used in place of Fluorapid film when the Patterson type B fluoroscopic screen is used. Since the spectral emission of this

screen falls between 5,200 and 6,000 A., high speed panchromatic emulsions, such as Agfa Ultra-speed, are satisfactory, as well as Fluorapid. For cine-fluorography, a reversible type film, such as Agfa Triple S Pan reversible, is suitable. Films of the Fluorapid type are, however, generally better than other films for both the photo-roentgen and the Patterson B screen and, in addition, have been found excellent for the photography of television images and cathode-tube tracings.*

TABLE II

TIME OF DEVELOPMENT IN AGFA NO. 30 DEVELOPER, 68 F	GAMMA FOR TIME-SCALE EXPOSURES, 5,400 K. LIGHT SOURCE, TANK DEVELOPMENT, AGITATION 5 SECONDS PER MINUTE	
Minutes	*Fluorapid*	*Ultra-Speed Pan*
2	0.95	0.65
4	1.5	0.85
6	1.88	0.95
8		1.0



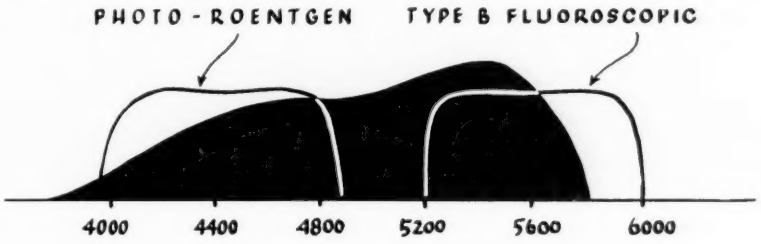
FILMS TANK DEVELOPED IN A-30 AT 68°F

METHODS, APPARATUS, AND MATERIALS

The actual making of a fluorogram consists simply of photographing the image of the fluorescent screen with a Leica, Contax, or other similar camera, equipped with an F/1.5 or F/2 lens, and loaded with a suitable film. In radiographic practice the roentgen-ray tube, the patient, the fluorescent screen, and the camera are placed in direct line with each other and properly spaced. The most simple arrangement, of course, is to place the camera on a firm support and direct it toward the screen. It is advisable, if not absolutely necessary, to place a thick piece of lead glass in front of the camera lens to absorb any of the direct roentgen rays that might fog the film. As previously mentioned, to avoid the effect of the direct roentgen rays Biesalski and Köhler[9] photographed a mirror

*By using a Contax camera, F/1.5 lens and Agfa Fluorapid film, cathode-ray oscillograph signals as short as 0.0003 second were recorded with good density. The size of the screen image on the film was about 3/16 inch. Previous to the actual exposure for recording the signal, an image of the dimly illuminated screen was produced on the film with a one second exposure.

image of the screen which permitted the placement of the camera outside the beam of direct radiation. However, this practice has not been followed by modern workers, such as de Abreu[14] and Hirsch,[11, 29, 30] who utilize a straight line system of almost identical specifications. The apparatus of de Abreu is mounted somewhat more elaborately than that of Hirsch, but the method of Hirsch is far more readily set up by the average clinical photographer. An illustration of his apparatus is shown in Fig. 3, *A* and *B*.



WEDGE SPECTROGRAM (SOLID)
OF FLUORAPID FILM
(TUNGSTEN)

Fig. 2.

A 35 mm. type camera* is preferable for fluorography in medical roentgenography for the following reasons: 1. The new type films, such as Agfa Fluorapid, are available only in this size. 2. Lenses of F/1.5 or F/2 relative aperture are standard equipment with several 35 mm. cameras. 3. The fluorograms in survey studies are made in great numbers and rapidly. This means that a camera which can make sequence exposures easily and rapidly is required, as well as a conveniently developed film. 4. The fluorograms are viewed by projection and projectors readily available and convenient to use. 5. The small film sizes are economical.

The lens of the camera should be of the highest quality in order to give an acceptably sharp image over the entire 24 by 36 mm. film area at wide open apertures. Hirsch lists the available lenses of F/1.5 relative aperture, or slightly faster, that can be considered. It would seem advisable to test any lens under consideration by a procedure such as that outlined by Gardner[31] in the Bureau of Standards Research Paper No. C 428. This publication not only provides a simple and accurate lens testing procedure, but also includes a carefully prepared lens test target.

It is not the purpose of the present discussion to delve into the qualities of various roentgen-ray equipment for fluorographic work. The general assump-

*Rather recently special 4 by 5 inch fluorographic equipment has been introduced for which ordinary high-speed roentgen-ray film has been found quite adequate. Such cameras are now employed by the army for group radiographic examinations, but as yet the 4 by 5 inch size has not won favor over the 35 mm. record for obvious reasons. Nevertheless, when lenses of high speed capable of covering a 4 by 5 inch field are generally available, this size is likely to surpass the 35 mm. method in popularity. For example, from a comparative study made by Plunkett, Weber, and Katz[36] it was concluded that 4 by 5 inch images were more suitable because of greater accuracy in reading; slightly better economy, resulting from fewer re-examinations; fewer 14 by 17 inch examinations to effect correct diagnosis; and less strain visually in examining 4 by 5 inch negatives than in the case of 24 by 36 mm. images.

TABLE III

WORKER	CAMERA AND LENS	TYPE OF SCREEN	DISTANCE		PEAK (KV.)	Ma.	TIME (SECONDS)	FILM	REFERENCE	VIEW
			CAMERA TO SCREEN	TUBE TO SCREEN						
Hirsch	Zeiss Sonnar 35 mm. F/1.5	Fluorazure or Patterson B	90 cm.	80 cm.	88	150	1/10 sec.	Agfa Fluorapid Eastman Special	No. 29, p. 6	Postero-anterior of chest
Hirsch	Zeiss Sonnar 35 mm. F/1.5	Fluorazure or Patterson B	90 cm.	70 cm.	96	80	4 sec.	Agfa Fluorapid Eastman Special	No. 29, p. 7	Gastrointestinal
Hirsch	Zeiss Sonnar 35 mm. F/1.5	Fluorazure or Patterson B	90 cm.	80 cm.	90	80		Agfa Fluorapid Eastman Special	No. 29, p. 7	Extremities
Hirsch	Zeiss Sonnar 35 mm. F/1.5	Fluorazure or Patterson B	90 cm.	60 cm.	90	160		Agfa Fluorapid Eastman Special	No. 29, p. 7	Skulls
Agfa Ansco X-ray technician	Leica 35 mm. F/2	Fluorazure or Patterson B	90 cm.	102 cm.	80	350	1/20 1/15 1/10	Agfa Fluorapid		Thin chest Normal chest Thick chest
de Abreu	F/1.5	Zinc Sulfide	90 cm.	60 cm.	80			Agfa Isochrom	No. 21	Chest
Holfelder	F/1.5	Zinc Sulfide	90 cm.	70 cm.	80			Agfa Isochrom	No. 32	Chest
de Abreu	Zeiss F/1.5	Patterson G. E. Siemen's Super-Astral	90 cm.	60 cm.	55 50	50 100	*0.2-0.3* *0.03-0.05*	Kodak Super XX Agfa Isopan I.S.S.	No. 14, pp. 365, 366	Adult chest Children
Schopper	Contax or Leica F/1.5-F/2	Hektophan (Auer)	15 times reduction	80 cm.	80	100	0.12	Agfa Fluorapid	No. 13	Chest
Böhme	16 mm.	Yellow-green fluoroscopic			80	30-50	24 to 32 frames per second		No. 16a	Cat thorax
Stewart and Ghiselin	Zeiss R-Biotar, F/0.85 5.5 cm. focal length 16 mm.	Special cyanide yellow green			100	110	16 frames per sec.	EK Super X	Nos. 16 and 33	Heart
Files	3¾ × 3¾ F/1.5	Patterson Fluorazure (Photo-Roentgen)		48 in.	55-80	400	1/10 adults 1/20 children	High Speed X-ray	No. 34	Chest
Steps	Zeiss Biotar F/1.4 35 mm. or 2¼ × 3¼	Neossal or Super-Astral	100 cm. (35 mm.) 77 cm. (2¼ × 3¼)	150 cm.	50-57	500 to 600	0.22	Agfa Isopan I.S.S.	No. 35	Chest

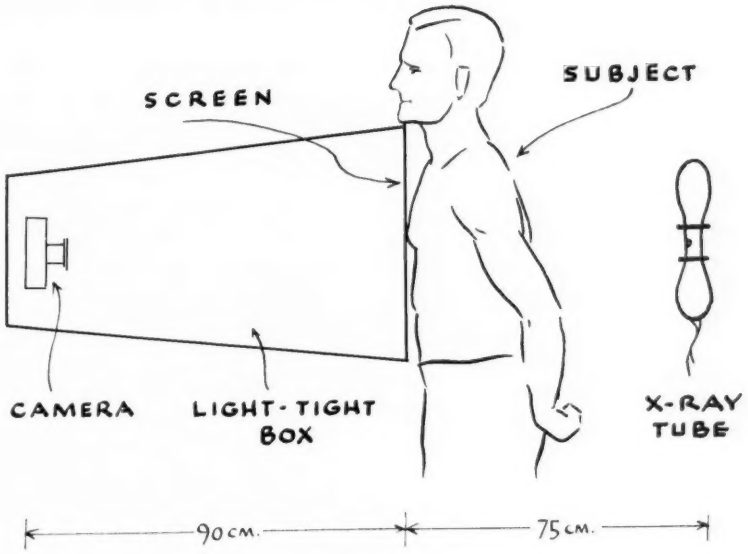


Fig. 3A.

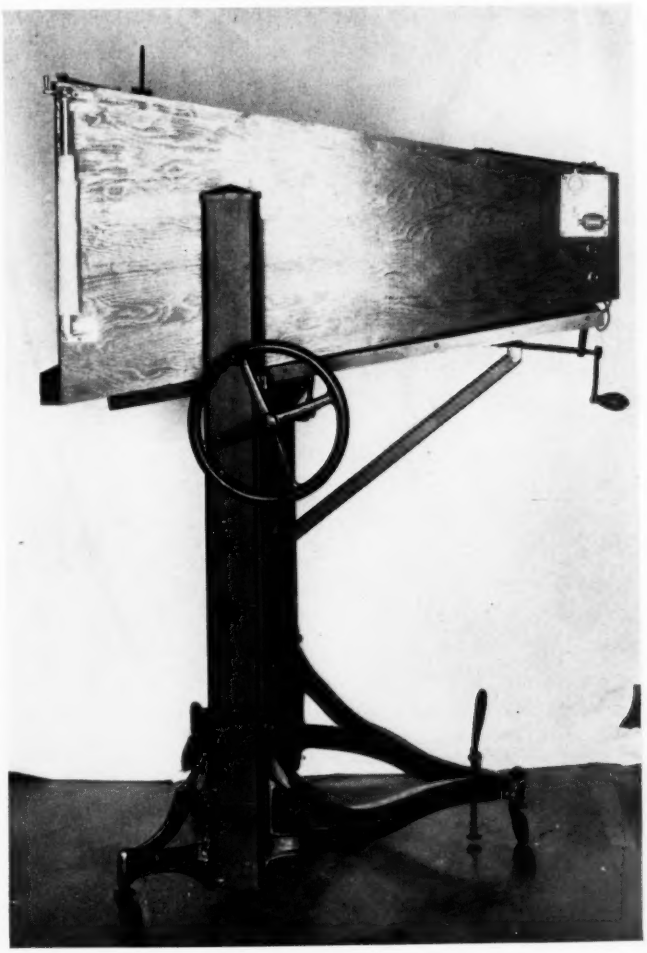


Fig. 3B.

tion is that any equipment of 150 Ma. capacity in conjunction with a roentgen-ray tube of the rotating anode type, capable of operating at 100 kv. loads, is adaptable to the process. For a guide in exposure, Table III has been prepared giving the data recommended by various workers.

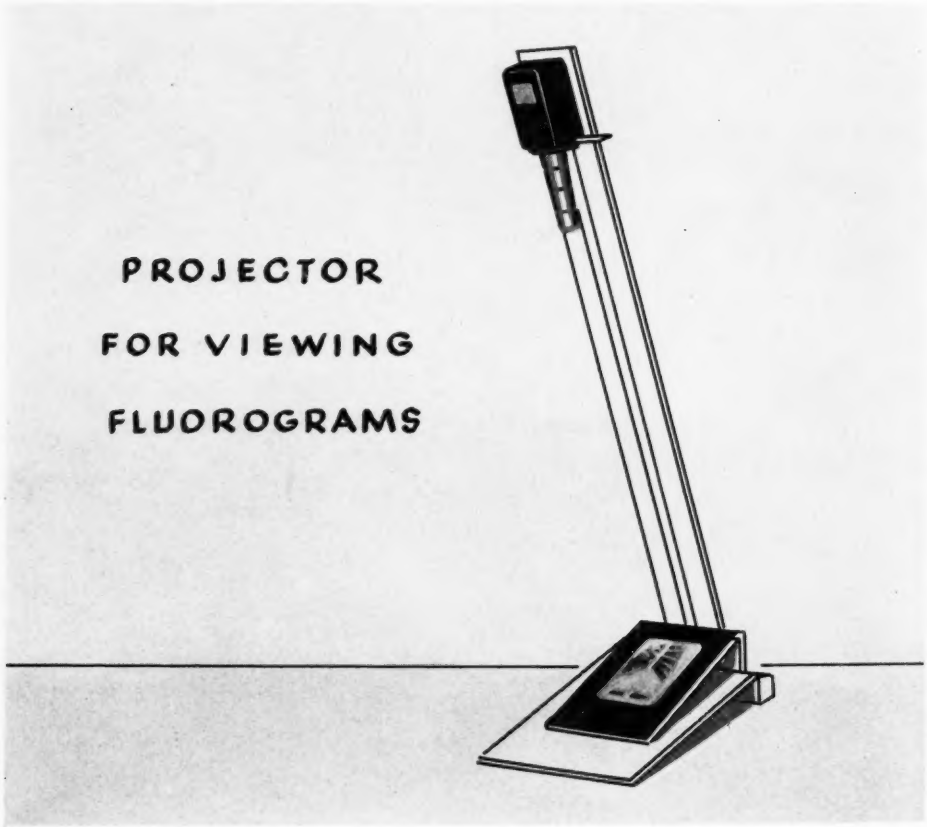


Fig. 4.

For the processing of the film, standard roentgen-ray developing and fixing solutions are satisfactory. The film can be looped around suitable racks if long lengths are employed, or small 35 mm. film developing tanks can be used for standard 5½ feet, 35 exposure lengths.

The fluorograms are projected when viewed, and for this purpose any number of adequate 2 by 2 inch slide projectors are available. A diagram of the projection arrangement used by Hirsch is shown in Fig. 4.

A convenient magnification of the fluorogram does not yield an image as large as the screen image, but if a 6.8 times enlargement is used, the size will be 15 per cent smaller than half size. For the average chest, this 15 per cent undersize just corrects for the distortion introduced by the use of a 30 inch target-screen distance and accordingly gives a true half-size view.

CONCLUSIONS

The advantages of fluorography in medical roentgenography are, chiefly, simplicity and economy in conducting mass examinations. The economy comes

from the lower cost of small-sized film and subsequent economies in developing and filing. The cost of the film is approximately 2 per cent of that of 14 by 17 inch roentgen-ray film. Perhaps the best discussion on the economy and also on the advantages of fluorography that has been presented is contained in the article by Hirsch,[29] to which reference can be made. A vital comparison between fluoroscopic examination and the fluorographic method made by Hirsch is that 350 fluorograms can be read an hour while only 250 fluoroscopic examinations can be made a day by one person. Not only is the process more rapid than direct examination, but it is more dependable, and in addition furnishes a permanent record. Of course, fluorograms cannot compete with large-sized radiographs with respect to image detail and general applicability. However, fluorography certainly has its place in preventive medicine. It is a process with which every clinical photographer should become acquainted.

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the Patterson Photo-roentgen or Fluorazure fluoroscopic screen has a definite afterglow. These screens as used in fluorography do not have a sufficiently persistent afterglow to influence the results. The persistence of the afterglow is in relation to the excitation energy received by the screen. A heavy dosage of roentgen rays or daylight would cause an afterglow as long as a half hour. This afterglow can be quenched by exposing the screen to deep red or infrared radiation. (See also Fluorescence and Phosphorescence, E. Hirschlaff, New York, 1939, Chemical Publishing Co.)

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SIMPSON MEMORIAL INSTITUTE
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DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

STERNAL MARROW, Changes During the First Week of Life. Correlation With Peripheral Blood Findings, Shapiro, L. M., and Bassen, F. A. Am. J. Med. Sc. 202: 341, 1941.

Peripheral blood studies and sternal puncture examinations were made on 35 normal full-term infants in the first twenty-four hours of life and again one week later.

The peripheral blood findings agreed with those of many other investigators. The authors noted initial high levels of red blood cells, hemoglobin, white blood cells, and reticulocytes. There was a moderate decrease in red blood cells and hemoglobin, a sharp fall in white blood cells and reticulocytes, and a change in the differential from myeloid to lymphocytic predominance at the end of the week.

The most striking finding in the bone marrow was a marked drop in the erythroid elements at the end of the first week of life. This finding, reflected in the peripheral blood by the decreasing reticulocytes, is evidence of curtailed production of new red blood cells. Consequently, the authors offer the idea that the fall in red blood cells and hemoglobin, which takes place after birth, is primarily the result of physiologic disintegration of the superabundant red blood cells carried over from fetal life in the presence of diminished erythropoiesis.

CEREBROSPINAL FLUID, Protein Values Determined by Tyrosine Equivalent Method, Marron, T. U. Am. J. Med. Sc. 202: 330, 1941.

Total proteins have been determined by the tyrosine equivalent method on 550 cerebrospinal fluids taken from persons in health and disease.

The upper limit of normal values has been found to be 46.0 mg. per 100 c.c. The ranges for pathologic values agree well with generally accepted figures in the literature.

The average protein values for females is less than that for males.

Dehydration does not appreciably affect the protein concentration.

TUBERCLE BACILLI, Demonstration of, by Fluorescence Microscopy, Richards, O. W., Kline, E. F., and Leach, R. E. Am. Rev. Tubere. 44: 255, 1941.

Procedure:

1. Make direct smears of sputum, or of sputum concentrated on glass slides, and fix by heat in the usual manner.

2. Stain for two or three minutes with a solution of 0.3 per cent auramine in 3 per cent carbolic acid solution at room temperature.

3. Wash with water.

4. Decolorize with a solution of 0.5 per cent concentrated hydrochloric acid and 0.5 per cent sodium chloride in 70 per cent alcohol for one minute, pour off, and add fresh decolorizer for two additional minutes.

5. Rinse, dry, and examine.

Smears should be made preferably with fresh sputum. If a preservative is desirable, phenol will be more satisfactory than cresol, which reduces the number of organisms and causes an interfering fluorescence. Autoclaving will greatly reduce the number of organisms seen.

This staining technique is neither a painstaking nor a critical one. Almost any concentration of auramine will give the desired result; as little as 0.05 per cent of dye may be used. The concentration of phenol may be reduced. Satisfactory results have been

obtained with 0.5 per cent auramine in 2 per cent phenol. The stain originally recommended was 0.1 per cent auramine in 5 per cent phenol. The auramine is dissolved with gentle heat, but the stain becomes cloudy on cooling. Cloudy solutions are just as satisfactory as clear ones, but should be shaken just before use. The stain, after a number of weeks, gradually loses some of its fluorescent properties. Smears of known positive sputa should be stained and examined occasionally as a check on the keeping qualities of the dye.

Almost any of the standard decolorizing agents mentioned for decolorizing tubercle bacilli, such as 3 per cent hydrochloric acid, 5 per cent nitric acid, or even 25 per cent sulfuric acid, may be used. These stronger reagents require more careful timing; most of them decolorize in from twenty to thirty seconds. The slower, three-minute period decolorizer is recommended.

The newly designed equipment for fluorescence demonstration of acid-fast organisms has the following advantages over older methods for examining smears of sputum and other material for the presence of tubercle bacilli:

(a) The method stains more acid-fast bacteria than does the Ziehl-Neelsen technique.

(b) Examinations can be made with high dry objectives that cover fields which are larger than those covered by oil-immersion lens as well as being simple and more convenient for use.

(c) A greater contrast is obtained between the stained organism and other material present on the slide.

MONONUCLEOSIS, Infectious, Observations on, Warren, E. W. Am. J. Med. Sc. **201**: 483, 1941.

The authors present evidence of the following phenomena to be found in infectious mononucleosis: Forssman heterophile antibodies are probably increased prior to the development of the heterophile antibodies of this disease and possibly are essential for their appearance. The typical lymphocytes of infectious mononucleosis are frequently found in disease entities in which a positive Paul-Bunnell has not been obtained. Only when the proportion of these abnormal cells is considerable does one find an elevated heterophile titer.

CL. WELCHII, Rapid Identification of, by the Nagler Reaction, Hayward, N. J. Brit. M. J., May 31, p. 811, 1941.

The production of a turbidity in human serum by the toxins of growing *Cl. welchii* (Nagler reaction) is a valuable aid to the rapid identification of the bacillus for diagnostic purposes. Excellent results are obtained with a mixture of equal parts of human serum and Fildes broth incubated anaerobically. The seeding of a single *Cl. welchii* colony into such a mixture produced a strong reaction in 80 per cent of the tests after one to two days' incubation. The reaction is specifically inhibited by antitoxin. Feeble reactions are not unequivocally indicative of *Cl. welchii*, since they are given by certain other species of anaerobic spore-bearer. Human serum-Fildes agar provides a means of detecting *Cl. welchii* on plates within twenty-four hours of taking a swab.

FUSOSPIROCHETOSIS, Recovery of the Causative Organism From the Blood, Williams, R. H. Arch. Int. Med. **68**: 80, 1941.

The recovery of fusiform bacilli and spirochetes, of the type found in Vincent's angina, in cultures of the blood of 2 patients is recorded. Fusospirochetes were recovered eleven times from one patient and once from the other. Successful subcultures of the organism were made repeatedly. The organisms were slightly pathogenic for rats.

In the 2 patients fusospirochetemia was associated with an illness characterized by the sudden development of fever, prostration, headache, myalgia, and migratory arthritis. In one patient splenomegaly developed, with jaundice, a hemorrhagic eruption, and acute pleuritis. The source of infection is considered to be the tonsils in one instance and the bite of a rat in the other.

EOSINOPHILIA, Extreme, and Leukocytosis, Bass, M. H. Am. J. Dis. Child. 62: 68, 1941.

Three cases in which the disease was characterized by general adenopathy, leucocytosis, and extreme eosinophilia that occurred in childhood are reported.

In one case the patient had roentgenographic evidence of miliary pulmonary infiltrations, which persisted for several years but finally disappeared.

The leucocytosis, and especially the eosinophilia, remained long after the fever had disappeared. The course of the disease is chronic, but it may end in complete recovery.

In one case death occurred from intercurrent illness during the first year of observation. In the second case the patient was kept under observation for several years and finally recovered completely. In the third case, in which the patient is still being followed, there was a febrile period, but the child is now apparently in good health; the total leucocyte count has returned to normal, but a marked increase in eosinophilic cells still persists.

Data from 4 cases, reported from Havana, in which the disease resembled that in these cases are cited. The 7 cases resemble each other closely and appear to be examples of a clinical entity.

The relation of this syndrome to other pathologic states in which there are extreme leucocytosis and eosinophilia is discussed, and it is shown that the syndrome fails to fit into the category of any of the previously described conditions. Although the cause of the syndrome is unknown, it is most probably some type of chronic infection.

SULFANILAMIDE, Erythrocyte Fragility Changes Produced by, Antopol, W., Goldman, L., and Sampson, W. L. Am. J. Med. Sc. 202: 163, 1941.

The administration of sulfanilamide to rats results in an increased resistance of the red blood cells to hemolysis in hypotonic saline solution, and in splenomegaly with the histologic picture of active hyperemia such as seen in hemolytic anemia.

CORONARY ARTERY OCCLUSIONS, Incidence and Localization of, Schlesinger, M. J., and Zoll, P. M. Arch. Path. 32: 178, 1941.

More than one-half of the points of occlusion in the coronary arteries are overlooked by ordinary dissections.

Most zones of occlusion of the coronary arteries are less than 5 mm. in length and are, therefore, easily overlooked.

Occlusions are as numerous in the right coronary artery as in the left descending coronary artery.

There is no relationship between the manner of branching of the coronary arteries and the localization of occlusions therein.

The majority of coronary artery occlusions are found within 3 cm. of the mouths of these vessels.

ITEM

Urology Award

The American Urological Association offers an annual award "not to exceed $500.00" for an essay (or essays) on the result of some specific clinical or laboratory research in urology. The amount of the prize is based on the merits of the work represented, and if the Committee on Scientific Research deem none of the offerings worthy, no award will be made. Competitors shall be limited to residents in urology in recognized hospitals and to urologists who have been in such specific practice for not more than five years.

Essays shall be in the hands of the Secretary, Dr. Clyde L. Deming, 789 Howard Avenue, New Haven, Conn., on or before April 1, 1942.